Proposed Methodology for Specifying Atrazine Levels of Concern for Protection of Plant Communities in Freshwater Ecosystems

Report To:

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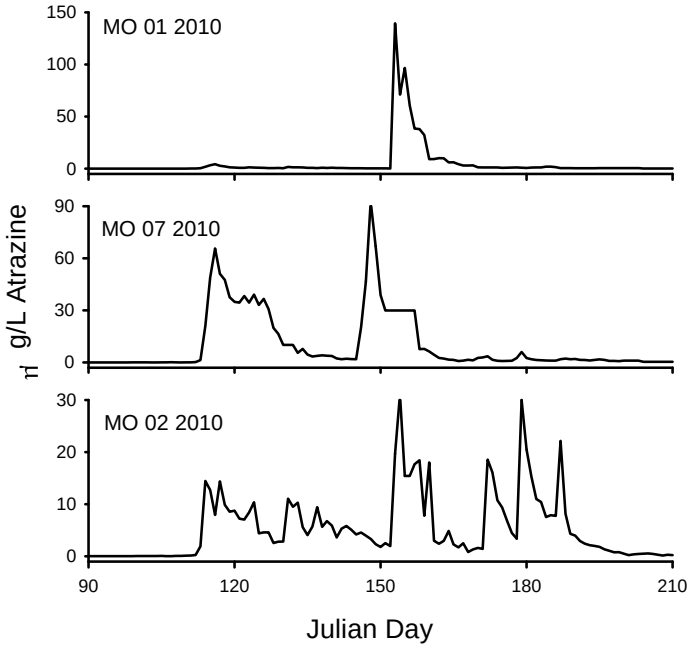
1. INTRODUCTION

This document describes proposed methodology for setting a level of concern (LOC) for atrazine in natural freshwater systems to prevent unacceptably adverse effects on the aquatic plant communities in those systems. Effects on humans and possible endocrine-disruption in aquatic vertebrates are subjects of separate efforts, and some implementation issues for aquatic plant community atrazine risk assessment are also described elsewhere. This first section defines the problem being addressed and describes a general framework for setting the LOC.

1.1 Requirements for the LOC Methodology

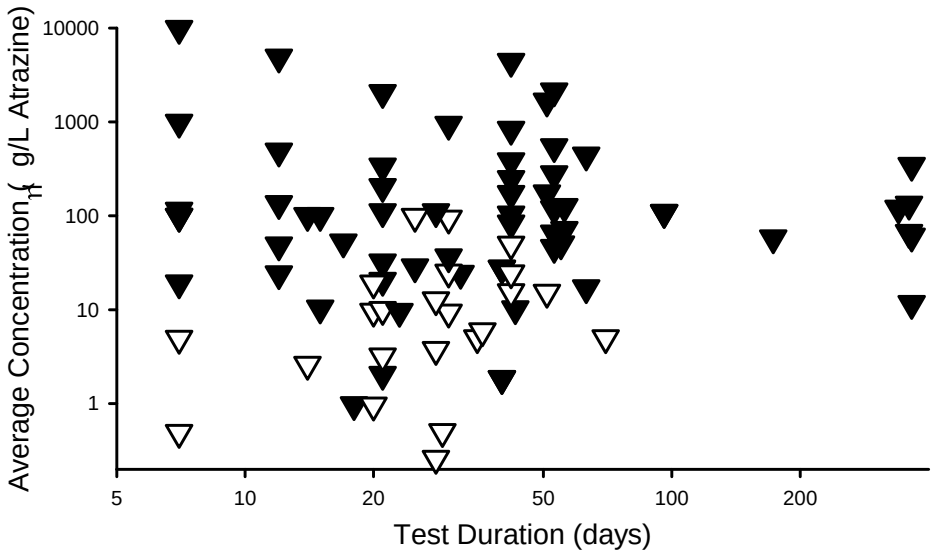
The LOC methodology must address the types of atrazine exposures occurring in natural ecosystems for which risk is to be assessed. Atrazine enters natural freshwater systems primarily in rainfall-driven runoff, resulting in highly variable and episodic exposures that depend on rainfall distribution, atrazine application patterns, topography, and soil properties. Figure 1 provides example time-series of atrazine exposures during 2010 in three Missouri streams, measured as part of a monitoring program being conducted to satisfy risk evaluations required under the 2003 interim reregistration of atrazine. These examples illustrate substantial variation in exposure patterns, and thus the need for the LOC methodology to address the relationship of effects to time, including high concentrations with limited durations, multiple events, and prolonged, variable exposures at low to moderate concentrations. The top and bottom series have similar average concentrations but very different peaks, underscoring the issue of the comparative risk of short, intense exposures to more prolonged exposures at lower concentrations.

Figure 1. Examples of atrazine exposure time-series in natural freshwater systems.



Regarding effects of concern, this LOC methodology will addressthe productivity and composition of aquatic plant communities. Although atrazine has been the subject of many toxicity tests on individual aquatic plant species and although such tests are often used to assess risk to aquatic plants (e.g., Solomon et al. 1996, Giddings et al. 2000), they will not be used directly for that purpose in this methodology. Instead, plant community responses documented in experimental aquatic ecosystem studies will serve as the foundation for defining exposures causing effects of concern on these communities. Figure 2 summarizes an evaluation of such studies conducted by the U.S.EPA's Office of Pesticide Programs (OPP) Environmental Fate and

Figure 2. Effects of atrazine on experimental ecosystems as a function of exposure duration and average concentration. Closed triangles denote adverse effects, open triangles no effects.



Effects Division (EFED) (U.S.EPA 2011). In Figure 2, each experimental ecosystem treatment is characterized by the duration over which effects were assessed, the average atrazine concentration over this duration, and whether there were unacceptably adverse effects on the plant community. For each point on Figure 2, Appendix B of this report provides more complete exposure information, the effects designation, and a literature citation; other information on the analyses of these studies can be found in U.S.EPA (2011). It should be emphasized that a fundamental assumption in using such experimental ecosystem data is that they collectively describe a relationship of effects to exposure that is relevant to the probability of effects (i.e., risk) occurring in natural freshwater systems. In other words, it is assumed that natural aquatic plant communities will generally react adversely if subjected to the same atrazine exposures that elicited adverse effects in the experimental ecosystem studies. This assumption is inherent in any assessment that extrapolates toxicity experiments to the field, and the use of experimental ecosystems arguably provides a better basis than do single-species toxicity tests.

Figure 2 illustrates three important requirements for the LOC methodology:

(1) Diversity among the experimental approaches precluded characterizing each experimental ecosystem treatment with a comparable, quantitative measure of effect. Therefore, LOC characterizations must rely on a binary (acceptable vs. unacceptable) characterization of effect.

(2) Although the exposures that resulted in adverse effects are somewhat separated from those that did not cause adverse effects, substantial overlap exists between these two groups, especially in the 10-20 µg/L range. This variability is presumably due to the combined effect of: differences in the nature of the experimental systems; differences in the experimental design and the endpoints measured; and random variability of the response of any given system. The methodology must address how to specify an LOC within such variability.

(3) The LOC methodology must address the relationship of effects to time. This is important not only because of the variability of field exposures shown in Figure 1, but also because of the widely different durations of the experimental ecosystem exposures in Figure 2 and the variability of exposures within these durations not shown in Figure 2 but provided in Appendix B. Because the diversity of the endpoints and test systems precludes any definitive empirical assessment of time dependence (e.g., Figure 2 does not provide information on the relationship of the same endpoint to exposure duration), this must be addressed in the formulation of the extrapolation methodology below.

1.2 General Framework for the LOC methodology

The key issue that this LOC methodology must address is how to relate aquatic plant community effects elicited in an experimental ecosystem by a particular atrazine exposure time-series to markedly different time-series in other experimental studies or natural systems. If all exposures of interest had the same shape (i.e., the same exposure duration and the same relative changes in concentration within that duration), the LOC could be based on the relationship of effects in the experimental studies to any convenient measure of exposure. However, the markedly different exposure shapes discussed above preclude such a simple approach, and there is thus a need for a method to translate any exposure time-series to a "common currency" that integrates time and concentration into an index of the relative total severity of effects from the exposure. This "effects index" serves only as a relative measure of effect because the experimental ecosystem effects define the absolute levels of concern. Text Box 1 further defines

Text Box 1. The nature and purpose of the "effects index".

To further clarify the nature and purpose of the "effects index", consider a simple hypothetical example in which the results from a single experimental ecosystem study must be used to assess risk to an identical ecosystem, but for an exposure with a different shape. For this example, the experimental ecosystem study is specified to (a) involve constant atrazine exposure over 30 d at several concentrations and (b) demonstrate that 20 µg atrazine/L constitutes an LOC based on the magnitude of effects elicited. However, this concentration-based LOC applies only to constant, 30-d exposures, whereas the exposure of interest is specified for this example to be a 10-d exposure at 100 µg atrazine/L. The basic question is whether this more intense (5x higher) but more brief (3x shorter) exposure should be considered worse than the 30 d LOC concentration, provided the effects are assessed in the same manner and over the same time period as in the original study.

A very simple "effects index" for this would assume that effects increase linearly with both concentration and time, so that the effects index could be the area under the exposure time-series, measured in "ppb-days" (note: this effects index definition is provided only to illustrate the concept – the actual methodology should consider the nonlinearity of effects versus exposure) The LOC for this effects index would therefore by 600 ppb-days (20 µg/L x 30 days) based on the experimental ecosystem study results. This effects index-based LOC is exceeded by the effects index value of 1000 ppb-days (100 µg/L x 10 days) for the new exposure of interest.

This effects index is a relative measure in that it has no inherent absolute meaning for risk except when calibrated to the experimental ecosystem results. Its use is only for translating any exposure time-series to a common scale of comparison, so that the LOC of 600 ppb-days can be used to judge any other exposure of interest, provided the exposure is for a system to which the experimental ecosystem is relevant.

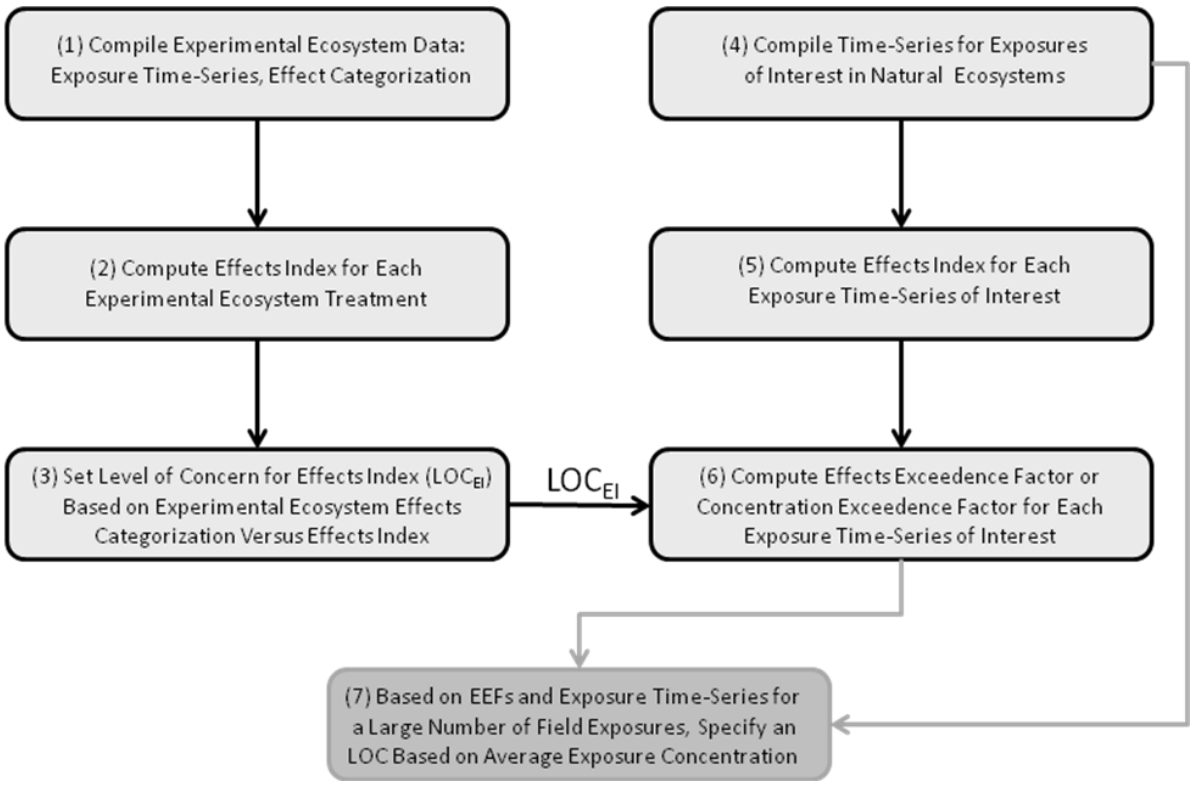
and discusses this concept of an effects index.

The effects index proposed for the LOC methodologywill be described in a later section. For discussing the assessment framework here, it is only necessary to assume the existence of an effects index appropriate for comparing the relative severity of different exposure time series. Figure 3 provides a schematic of an assessment framework using such an effects index.

The process starts (Box 1) with compiling relevant experimental ecosystem data, categorizing each treatment as to whether there was an effect or not and specifying the exposure time-series for the treatment. This step is not a subject of this report, but rather, as noted above, is addressed in U.S.EPA (2011). The effects index is then calculated (Box 2) for each experimental ecosystem treatment, providing the "common currency" to compare the severity of each exposure. The relationship of the binary experimental ecosystem effects to the effects index is then examined (Box 3) to set a level of concern for the effects index (LOC_{EI}), based on the probability of eliciting an effect (i.e., risk).

This LOC based on experimental ecosystem data and on an effects index is applied to exposures in natural systems as follows. Exposure time-series are compiled for the various exposures of interest in natural ecosystems (Box 4) and the effects index for each exposure is computed (Box 5). Risk is characterized (Box 6) by dividing the effects index by the LOC_{EI} to compute the "effects exceedence factor" (EEF). The EEF indicates whether the LOC is exceeded (i.e., EEF>1) and by how much. The EEF thus represents a risk quotient approach, but this

Figure 3. Assessment framework for risk of atrazine to aquatic plant communities, based on experimental ecosystem results and an effects index for comparing different exposure time-series.



different terminology is used here to distinguish this effects-based quotient from concentration-based risk quotients commonly used.

Risk can also be characterized by what is termed the "concentration exceedence factor" (CEF) in Box 6. This factor is based on iterative calculations to determine the multiplicative factor by which the exposure must be decreased so that the effects index exactly equals the LOC_{EI}. As for the EEF, a CEF indicates whether the LOC is exceeded and by how much, but on a concentration scale rather than an effects scale. This could have some advantage in determining remediation needs or, conversely, determining how far exposures are below levels of concern. However, this is an approximate measure for such purposes, because the CEF is premised on the same multiplicative factor applying to the entire concentration time-series.

Box 7 and the associated gray arrows in Figure 3 represent a final step in the assessment framework, whic isnot addressed in this document. It would be desirable for LOCs to be on a concentration scale rather than an effects scale so that they relate more easily and directly to exposure monitoring data. In Box 7, the relationship of EEFs to an average exposure concentration for a large number of existing exposure time series is examined to determine an LOC based on this average concentration, and which then can be applied to new exposure time-series for which the effects index is not directly computed. Developing such a concentration-based LOC from the effects index-based LOC is being addressed separately by EFED.

Finally, it should be emphasized that the only site-specific factor intended to be addressed in this LOC methodology is the exposure time-series. The methodology is not intended to address other site-specific factors, such as physicochemical conditions and the nature of the biological community. Addressing such conditions is not feasible from a standpoint of both effort/cost and knowledge of their influence on atrazine effects. Rather, this method will be generic in that any site with the same atrazine concentration time-series will be assessed as having the same risk.

2. PLANT ASSEMBLAGE TOXICITY INDEX

2.1 Potential Effects Indices

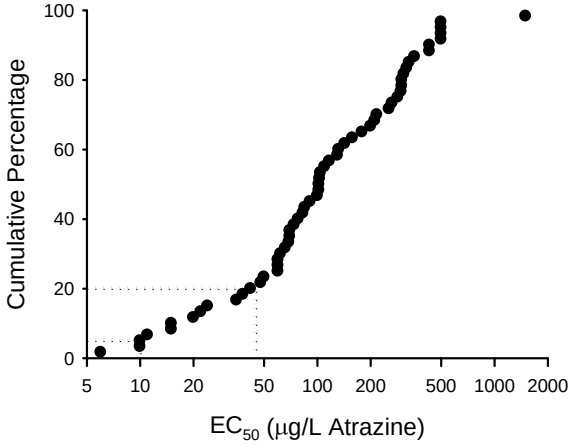
There are various possibilities, with differing complexities, for calculating an effects index to serve in the assessment framework of Figure 3. For illustrative purposes only, Text Box 1 assumed that effects increased linearly with both concentration and time, leading to an effects index of ppb-days. To actually apply this simple, linear model *a priori* is not justified. Rather, the effects index should incorporate relevant and appropriate ecotoxicological relationships.

At the other extreme of complexity are community simulation models that address not only the immediate impact of atrazine on plant community primary production, but also consider the ramifications of this on plant community dynamics throughout a growing season. Early efforts for developing an LOC methodology considered the use of the Comprehensive Aquatic Simulation Model (CASM), but determined that this model was not suitable for the purposes here (U.S.EPA 2009, Erickson 2009). This model does not provide any clear, validated, substantial added-value beyond the immediate response of plant community growth, entails extensive data and parameterization needs that were not completely satisfied, and involves considerable uncertainty. CASM is more suited for focused site assessments - requiring considerable resources for model development and application and involving a completely different assessment framework.

A community simulation model such as CASM applies information from atrazine toxicity tests on individual plants species to calculate the direct (primary) impact on the plant community being simulated, but then also considers the secondary (indirect) ramifications on plant community dynamics. The direct, primary impact was determined to be much more important for assessing the relative impact of different atrazine exposure time-series (i.e., the purpose of the effects index) than are those secondary impacts (U.S.EPA 2009). Thus, the approach pursued here was to base the effects index just on this primary impact, avoiding various uncertainties and complexities in the community model.

The need here is therefore to use the collective information from toxicity tests on individual plant species to provide a measure of direct impacts of atrazine on plant communities. To this end, past assessments of the risk of atrazine to aquatic plant communities (e.g., Solomon et al. 1996; Giddings et al. 2000) have generally summarized the results of a toxicity test as a median effect concentration (EC_{50}), the concentration causing a 50% decrease in some measure of growth over the duration of the test. Average EC_{50}s for each species are then used to describe a species sensitivity distribution (SSD), the cumulative percentage of species with EC_{50}s less than a

Figure 4. Example of aquatic plant SSD based on data from Giddings et al. (2000).



certain value (e.g., Figure 4). SSDs are typically applied by addressing what percentiles are exceeded by an exposure. For example, in Figure 2, an exposure of 10 μg/L would be below the EC_{50}s of 95% of the species and an exposure of 45 μg/L would be below the EC_{50}s of 80%.

However, such SSDs have major shortcomings, especially for addressing the types of exposures in Figure 1:

(1) SSDs based just on EC_{50}s provide limited information on the overall toxic impact to the assemblage of species used for the SSD. For example, the 5th percentile in Figure 4 only describes the concentration at which the growth of a particular species is reduced by 50%. No information is provided on how much greater effects are for this species at higher concentrations, or how much lower effects are at lower concentrations. For other species, no information is given other than that their EC_{50}s are less than or greater than the LOC. Much more information regarding effects is contained within the toxicity test data, but how should it be used?

(2) SSDs such as in Figure 4 also do not address the issue of time. How should effects be described for longer or shorter exposures and, especially, exposure concentrations that fluctuate? If the LOC is simply applied to the peak exposure, the exposure time-series in the top panel in Figure 1 would be considered of most concern, but toxic impact would probably be higher for the middle time-series and perhaps as much for the lower time-series, because of the more prolonged and multiple exposure periods. How should total impact be assessed over an entire time-series?

(3) Although the EC_{50}s in Figure 2 all describe plant growth in some fashion, growth is measured in a variety of ways (final plant biomass, net change in biomass, growth rate, oxygen evolution, carbon fixation, plant length, cell numbers, changes in chlorophyll) and over a wide range of exposure durations and conditions, such that these EC_{50}s can have greatly different meaning regarding actual plant sensitivity. The spread of values in the SSD might therefore be due to differences among test endpoints as well as differences among species. Such inconsistency in the meaning of EC_{50}s will cause any LOC from the SSD to have uncertain meaning.

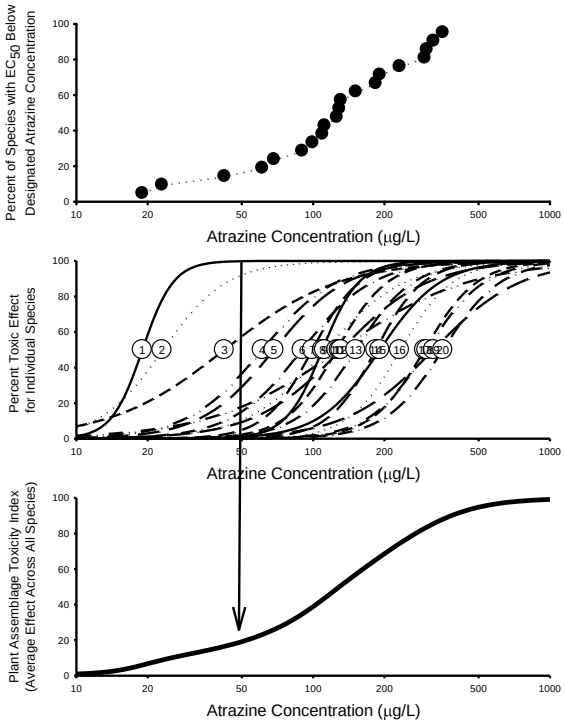
2.2 Definition of the Plant Assemblage Toxicity Index

To quantify the overall effect of atrazine on an assemblage of plant species of interest, the effects index proposed here is the "Plant Assemblage Toxicity Index" (PATI). PATI is a simple extension of the SSD concept that (a) considers the entire growth inhibition vs. concentration curve (“toxicity relationship”) for each plant species and (b) determines the average effect level across all species (the "assemblage") at each concentration. Figure 5 illustrates how toxicity relationships addressed in Appendix A can be used in this way. The middle panel shows overlapping toxicity relationships for 20 plant genera. In the top panel, the EC_{50}s for each genus are used to create a traditional SSD – just the cumulative percentage of the EC_{50}s. For the bottom panel, the average magnitude of effect across all species at each concentration is used to create the PATI distribution. At 50 μg/L, the average effect over all genera is 19%, providing the PATI value in the bottom panel (arrow). Thus, rather than just providing the percentage of species that have an EC_{50} below some concentration (50 μg/L corresponds roughly to the 16th percentile of on the SSD), PATI describes the percent reduction in plant production for the entire assemblage (weighting each species equally). Although the shape of the PATI curve is similar to

that of the traditional SSD curve, it provides more information on the total impact on the plant community and allows better comparisons between different exposure concentrations.

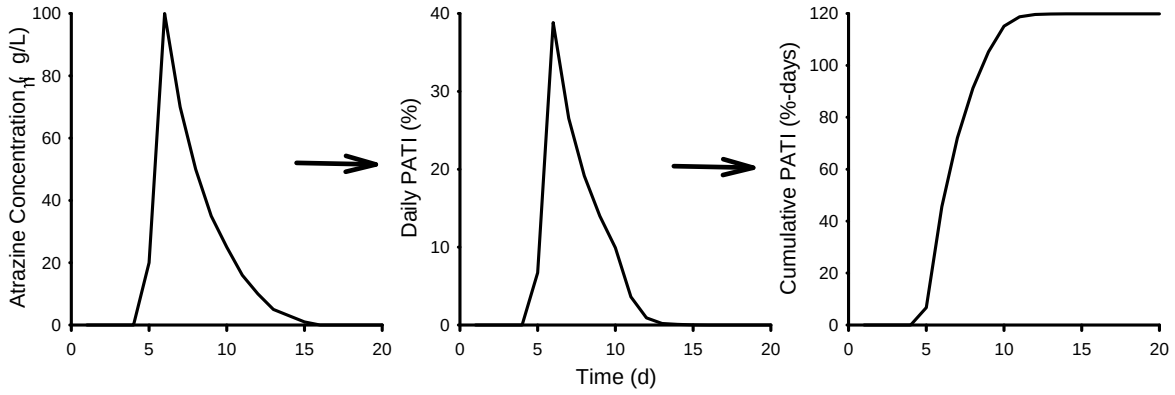
However, the definition and calculation of PATI illustrated in Figure 5 is not yet complete because it does not address the issue of time. For a time-series of daily concentrations, there would need to be separate calculations for each day to generate a time-series of daily PATI values. Because of the rapid recovery of growth rates in toxicity tests when exposures are terminated (e.g. Abou-Waly et al. 1991, Desjardin et al. 2003), daily PATI values need not consider residual toxicity from exposures on previous days, but rather only the toxicity for the current exposure.

Figure 5. Comparison of toxicity relationships for 20 plant genera (middle panel), the SSD of EC_{50}s for these genera (top panel), and the plant assemblage toxicity index (bottom panel, PATI = the average of the curves in the middle panel).



Because the effects index is intended to describe total toxic impact, the approach here to address time is simply to sum the daily PATI values to provide a "cumulative PATI". Figure 6 illustrates this – concentrations in the left panel are converted to daily PATI values (middle panel), which are then summed to provide the cumulative PATI values in the right panel. The cumulative PATI can also be viewed as the "area under the curve" of the daily values, this

Figure 6. Summary of PATI calculations. A concentration time-series (left panel) is converted to expected instantaneous or daily reductions in plant assemblage growth (middle panel), which is then integrated to provide a cumulative PATI value for the exposure (right panel).



area being a measure of the total toxic impact of the exposure.

The summation units of this cumulative PATI are analogous to the ppb-days discussed earlier or, more familiarly, with degree-days used to describe the total heating or cooling impact of seasonal weather. A fundamental aspect of such a summation is that a certain reduction in growth over 1 d is treated as having equal importance as: half that reduction persisting for 2 d; a quarter of that reduction persisting for 4 d; etc. Although this general time-dependence has not been demonstrated for actual aquatic ecosystems, it is observed for cumulative effects on biomass in single-species toxicity tests that maintain a constant level of effects on plant growth rate during the exposure period (e.g., Shafer et al. 1994).

This methodology uses a simple summation of toxic effects to provide an index for the relative toxic effects of different time-series on plant communities and deliberately does not address any further effects on plant community dynamics beyond short-term reductions in growth across the plant assemblage. As already noted, the basic PATI calculation is similar to the first step in community models such as CASM, which on each day calculates the toxic impact on the growth of various species – the fundamental difference being that PATI does not consider how this toxicity changes community composition through time. Nonetheless, community dynamics are driven on each day by the same growth reductions that are incorporated into PATI, so that PATI does embody the primary driving force for atrazine effects on plant communities. Even if community dynamics modify the relative severity of some time-series compared to that expected based just on PATI, these would be secondary effects and are not understood well enough to be satisfactorily addressed (U.S.EPA 2009, Erickson 2009).

However, this summation cannot be continued indefinitely, but rather is limited here to an "assessment period" that reflects risk management decisions about cumulative effects. For example, if two short atrazine exposures were separated by 90 d, a 120 d assessment period would consider them cumulative whereas a 60 d assessment period would not, this shorter period instead assuming that sufficient time had passed that the second exposure should be assessed independently of the first. The shorter assessment period would also avoid assigning concern to prolonged low exposures of uncertain, minor impact. For exposures with finite durations less than the assessment period, the summation would simply stop at the exposure duration. For exposures with durations greater than the assessment period, the summation would encompass the worst part of the exposure. For this report, this limit on cumulative toxicity will be designated with a subscript denoting the length of the assessment period (e.g., $PATI_{30d}$ denotes a 30-d assessment period). Without a subscript, PATI will refer to daily or instantaneous values, or the general PATI concept. The selection of the assessment period is addressed in Section 4.

2.3 Single-Species Plant Toxicity Test Data

Implementation of the PATI approach requires a compendium of the effects of atrazine on aquatic plants or statistical distributions describing these effects. Existing compendia of plant effects concentrations (ECs) (e.g., Giddings et al. 2000) have certain shortcomings regarding their applicability to risk assessment, which warranted reanalysis of existing single-species toxicity tests. This section describes: the shortcomings of concern; a new review and analysis of toxicity data; and a new compendium of plant toxicity information more suitable for calculating PATI and for conducting atrazine risk assessments.

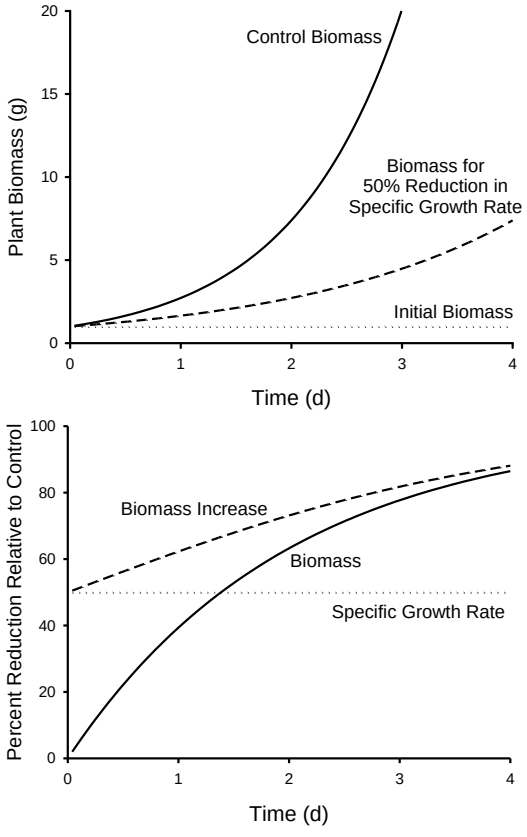
2.3.1. Issues in Interpreting and Applying Plant Toxicity Test Results

ECs from plant toxicity tests can vary widely in both value and meaning depending on how tests are conducted and analyzed. For microalgae, tests are usually conducted on cell suspensions under favorable (at least at test start) conditions of temperature, light, and nutrients. These tests can involve various measurement endpoints, including (a) actual biomass; (b) surrogates for biomass such as cell counts, cell volume, optical density, or chlorophyll content; and (c) indicators of growth such as oxygen evolution or radioactive carbon fixation. The period over which measurements are made can vary from several minutes to several weeks, and measurements might be reported at multiple times or only at the end of exposure. Biomass or biomass surrogates might be analyzed based on (a) biomass values at various times during the exposure, (b) biomass increase (growth) at various times, (c) the area under the growth time-series (AUC), and/or (d) specific growth rate (SGR)[1].

The meaning of an EC can be greatly affected by test duration and by whether it is based on absolute biomass, growth, or SGR. To illustrate this, Figure 7 provides a hypothetical example comparing growth when the control SGR (SGR_C) is 1.0/d to when a chemical exposure reduces the SGR to half of this value. The top panel shows the actual biomass vs. time in the control compared to the chemical exposure, while the bottom panel shows the percent reduction due to chemical exposure for SGR (constant at 50%), absolute biomass, and growth (biomass increase).

For growth, the treatment that is an EC_{50} for SGR will be an EC_{62} at 1 d, an EC_{73} at 2 d, and an EC_{88} at 4 d if the SGR_C is 1/d. For absolute biomass, this concentration would be an EC_{39}, EC_{63}, EC_{86}, respectively, at these times. For other values of SGR_C, more widely ranging ECs can occur. Using absolute biomass can result in particularly misleading ECs when growth rates are modest. For example, when control growth is just a doubling of biomass over the duration of the

Figure 7. Variation of plant growth effects with time and measurement endpoint. Top panel shows exponential growth for the control SGR (solid line) and one-half of the control SGR (dashed line). Bottom panel converts this to percent effect on biomass (solid line), on biomass increase (dashed line), and on specific growth rate (dotted line).



[1] The specific growth rate (SGR) =dB(t)/dt/B(t), where B is biomass and t is time. SGR is thus the fractional rate of change of biomass with time and has units of inverse time. If SGR is constant, the growth rate is exponential and $B(t)=B(0)\cdot e^{SGR\cdot t}$. Thus, if SGR is 1/d, this does not mean that the biomass will double in one day; rather the "compounding interest" of exponential growth will mean that biomass actually increases to 2.7 times the initial value – only over short periods will fraction growth closely adhere to SGR (e.g., 1% growth over 0.01 d).

test, an EC_{50} for absolute biomass actually represents no growth. Such issues with endpoint definition have been noted by others (e.g., Bergtold and Dohmen 2010) and are reflected in recent OECD guidelines.

Therefore, EC_{50}s reported for absolute biomass, growth, and SGR will differ from each other, and these differences will vary with exposure duration and the SGR_C. This is especially problematic when reports for toxicity tests just provide ECs, without sufficient information on absolute biomasses and/or SGRs as a function of time and concentration to calculate more consistent and meaningful measures of effect. Compendia that simply transcribe reported EC_{50}s can be describing a wide range of different effects, and assessments based on such compendia will be ill-defined.

Other factors make the meaning of reported plant ECs even less certain. As an algal suspension grows, the growth rate will decline because of nutrient depletion and self-shading. This departure from exponential growth will be most pronounced in the treatments with the highest growth rates (i.e., the control and low toxicant concentrations with little or no effect), so that the treatments with greater toxic effects might "catch up" as exposure duration increases, causing ECs for total growth to not decrease with time as much as they would without these limitations, or to even increase with time. In other words, the toxicity test actually can include stressors (nutrient/light limitations) in addition to the toxicant and that can confound the effects of the toxicant. In fact, some standard plant test protocols were originally designed to assess nutrient limitations, and the durations were selected to result in nutrient depletion (e.g., Miller et al. 1978). When used for toxicants, this type of study design can result in complicated growth dynamics and relationships that are difficult to interpret and apply. Tests can also have different photoperiods, which would also need to be considered in comparing ECs for growth (although ECs for SGR can be directly compared between different photoperiods).

Schafer et al. (1994) provide a noteworthy example of some of these problems. In a 10-d test in a flow-through system in which a constant control growth rate was maintained by replenishing the nutrient solution and periodically cropping biomass, they reported growth-based EC_{50}s to drop from 50 μg/L at 4 d to 20 μg/L at 7 d to 10 μg/L at 10 d. This is plausibly attributable to a constant relationship of SGR to concentration during these 10 d, so that a constant EC for growth rate translates into widely variant ECs for growth. These authors also reported an EC_{50} of 350 μg/L for a static, 3-d flask test, indicating much less sensitivity compared both to the flow-through systems and to photosynthesis measurements made in the first day of these static tests. This apparent lower sensitivity likely is due at least partly to a high initial cell density ($2 \cdot 10^5$ cells/ml), which would have resulted at 3 d in a cell density of $3 \cdot 10^8$ cell/ml if a SGR_C similar to that in the flow-through system had been maintained for the entire 3 d. Such a cell density would have resulted in both self-shading and nutrient depletion in the control, likely contributing to the apparent reduced sensitivity. Increases with time for growth-based ECs are evident in other studies in the review presented later, although the opposite can also be true, indicating additional complexities.

Changes in cell condition other than light and nutrient limitations might also affect ECs and their dependence on test duration. For example, chlorophyll content per cell can increase with time to compensate for reduced photosynthesis. Mayer et al. (1998) reported the

chlorophyll content of algal cells to increase by 10-fold in response to exposure to 200 μg/L atrazine. Such changes in the chlorophyll content per cell make the use of chlorophyll as a surrogate for plant biomass inadvisable, potentially misrepresenting toxic effects on biomass. For example, van der Heever and Grobbelaar (1996) reported effect concentrations in the same exposures to be about 2.5-fold higher when based on chlorophyll than when based on cell numbers or dry weight. Similarly, toxicants can alter cell volume and mass (e.g., van der Heever and Grobbelaar 1996), creating differences among ECs based on cell count, cell volume, and cell weight, although these differences are much smaller than those due to the influence of chlorophyll, test duration, nutrient depletion, and light limitations.

Although oxygen production and radiocarbon fixation are arguably closely linked to biomass production, ECs based on these measures can also pose interpretation problems:

(a) They are often done over such short durations that apparent effects might be reduced because of the time it takes to fully induce the effects of a toxicant, unless there is sufficient pre-exposure to the toxicant before the measurements are made. Fortunately, for atrazine, effects do appear to be induced quickly, such that EC_{50}s based on oxygen measurements with just several minutes prior exposure have been reported to be similar to those based on biomass measurements (e.g., Turbak et al. 1986).

(b) Short-term radiocarbon fixation rates can conceivably reflect gross or net photosynthesis (or a weighted combination of the two) depending on the disposition of the radioactive carbon in the organism. Williams et al. (1996) determined that radiocarbon fixation over short periods approximates net photosynthesis for good growing conditions (which would be expected in toxicity tests); therefore, radiocarbon fixation will be assumed in this review to represent net photosynthesis.

(c) Although oxygen production should parallel net photosynthesis, test methods using oxygen evolution measurements can involve extremes of oxygen concentrations that might affect photosynthesis and/or respiration – either high, supersaturated levels as oxygen increases from initial levels, or low concentrations due to the methodology involving an initial purging of oxygen. Studies with such extremes will not be used in this review because of uncertainty about their impacts.

(d) Even when the test is such that oxygen production or radiocarbon fixation are arguably good surrogates for biomass production, the time-scale of the measurements can affect their interpretation. Short-term values for oxygen production or radiocarbon fixation for an approximately constant mass of algae are analogous to the SGR, whereas measurements long enough for substantial growth to occur would be analogous to net cumulative growth, creating differences in the meaning of ECs similar to that for growth vs. SGR. In one study (Larsen et al. 1986), the situation was especially complicated because carbon-14 fixation was measured only during a short period at the end of a 24-h atrazine exposure, so that the measured fixation rate reflected ***both*** effects of the toxicant on the rate of carbon fixation per cell and the cumulative differences in cell density due to the preceding exposure.

Macrophyte tests can be less susceptible to the issues of exponential growth and limiting conditions discussed above. Many macrophytes grow slowly enough so that biomass increases

by only a few multiples during the tests. Duckweed tests show more rapid growth, but also usually do not reach biomass levels sufficient to suppress growth rates (frond crowding or nutrient depletion). However, the general issues raised above for microalgae should still be considered in the interpretation of macrophyte tests and the definition of their ECs. For example, reduced photosynthesis can result in elongation of plant shoots with little or no biomass increase, so that shoot length can be a poor surrogate for biomass changes (e.g., Fairchild et al. 1994, 1998). In addition, some macrophyte tests involve rhizomes, which contain resources to temporarily support growth that might obscure toxic effects, again making length a questionable measure and even making weight problematic if only shoot biomass is measured. Furthermore, if test protocols with cuttings result in slow growth (e.g., due to the absence of rooting), variability can make it difficult to quantify toxic effects and/or make such toxic effects of uncertain relevance to the field. Finally, use of oxygen in interpreting growth of some vascular plants might be confounded by gas exchanges to aerenchyma (air channels).

2.3.2. Review of Single-Species Plant Toxicity Tests

The inconsistency issues among single-species toxicity test ECs discussed above have not been adequately addressed in past reviews of atrazine toxicity (e.g., Solomon et al. 1996; Giddings et al. 2000) and might distort atrazine risk assessments. There was thus a need for better analyses of single-species plant toxicity tests with atrazine to produce EC compendia which are more consistent, providing a "common currency" that can be more legitimately compared among tests and applied meaningfully to risk assessments. The SGR was selected as this "common currency" because it reduces the dependence of ECs on test duration and is more directly applicable to addressing effects of time variable exposure. In addition, rather than simply compiling information on EC_{50}s, there was also a need for information on the entire EC vs. concentration curve, which is also inadequately addressed in previous compendia.

To this end, available single-species toxicity tests with atrazine were reviewed for information regarding exposure conditions and effects by the Great Lakes Environmental Center (Traverse City, MI) under support from the Office of Science and Technology of U.S.EPA's Office of Water (EPA Contract 68-C-04-006, Work Assignment 4-34, Subtask 1-16). Journal articles and reports identified by this review as containing potentially useful information were further analyzed by U.S.EPA to compile desired information on the relationship of SGR to atrazine concentration, using the following sigmoidal relationship (logistic equation):

$$SGR = \frac{SGR_C}{1 + e^{4 \cdot Steep \cdot \left(\log_{10} C_{ATZ} - \log_{10} EC_{50} \right)}} \qquad \textbf{(Equation 1)}$$

for which the parameters are the SGR-based EC_{50}, the steepness of the relationship of SGR vs. atrazine concentration ("*Steep*"), and the control SGR (SGR_C). Appendix A further discusses this equation and its use in the analyses, as well as (a) guidelines and procedures used in the EPA evaluations of toxicity tests and (b) a summary of each toxicity test reviewed. Table 1 provides the compilation of SGR EC_{50}, Steep, and SGR_C from these analyses.

Table 1. Compiled data regarding atrazine toxicity to aquatic plants. All data pertain to the specific growth rate (SGR) of the plant. Compilation includes the EC_{50} for the SGR, a steepness parameter for a fitted logistic relationship of SGR to atrazine concentration (Steep=-d(SGR/SGR_C)/d($\log_{10}(C_{ATZ})$) at the EC_{50}), and the control SGR (SGR_C) under the test conditions. Italicized EC50s denote values whose estimation required information on SGR_C and/or steepness from other studies. Appendix A provides more details on these data and analyses.

Genus	*SGR EC_{50} (µg/L)*	*Steep*	*SGR_C (d^{-1})*	*Reference*
CHLOROPHYTA (includes tested green algae)				
Ankistrodesmus	104	1.41	0.33	Burrell et al. 1985
	119			Larsen et al. 1986
Chlamydomonas	378	0.65		Kallqvist and Romstad 1994
	141		1.06	Schafer et al. 1993
	67			Larsen et al. 1986
	45			Hersh and Crumpton 1989
Chlorella	26	1.07	>1.4	Faust et al. 1993
	37			Hersh and Crumpton 1989
	91	0.47	0.26	Burrell et al. 1985
	557			Larsen et al. 1986
	480			Stratton 1984
Scenedesmus	***87***			Larsen et al. 1986
	300			Stratton et al. 1984
	39	***0.73***		Zagorc-Koncan 1996
Selenastrum	164	0.79	1.80	Mayer et al. 1998
			1.93	Radetski et al. 1995
	50	1.66	1.25	Caux et al. 1996
	100	1.50		Versteeg 1990
	131	0.62	1.75	Hoberg 1991A
	70			Turbak et al. 1986
	163	1.22	1.65	Roberts et al. 1990
	125	1.07	1.01	Gala and Giesy 1990
	110	0.90		Kallqvist and Romstad 1994
	201	0.79		Kallqvist and Romstad 1994
	236	1.01		van der Heever and Grobbelaar 1996
	223	0.61		van der Heever and Grobbelaar 1997
	101	1.61	0.97	Parrish 1978
	78			Larsen et al. 1986
Stigeoclonium	***317***			Larsen et al. 1986
Ulothrix	***159***			Larsen et al. 1986
CHROMALVEOLATA (includes tested diatoms, cryptomonads)				
Cryptomonas	494	1.15		Kallqvist and Romstad 1994
Cyclotella	462	1.22		Kallqvist and Romstad 1994
	100	0.67		Millie and Hersh 1987
	114	0.65		Millie and Hersh 1987
	225	1.00		Millie and Hersh 1987
Navicula	217	1.08	1.03	Hughes et al. 1988
CYANOBACTERIA (includes tested blue-green algae)				
Anabaena	70			Stratton 1984
	280			Stratton 1984
	470			Stratton 1984
	706	0.59	0.76	Hughes et al. 1988
	286			Larsen et al. 1986
Microcystis	164	1.25	0.55	Parrish 1978
	605	0.77		Kallqvist and Romstad 1994
Synechococcus	136	0.59		Kallqvist and Romstad 1994
ANGIOSPERMAE (includes tested vascular plants)				
Ceratophyllum	24	0.81	0.04	Fairchild et al. 1998
Elodea	65	0.38	0.07	Forney and Davis 1981
	<38		0.02	Fairchild et al. 1998
	204	0.52	0.09	Hoberg 2007
Hydrilla	118	0.99		Hinman 1989
Lemna	202	1.24	0.24	Hoberg 1991B
	93	1.33	0.25	Hoberg 1993B
	49	1.71	0.23	Hoberg 1993C
	115	0.42	0.21	Fairchild et al. 1998
	224	1.14	0.21	Hughes et al 1988
	95			Kirby and Sheehan 1994
	90	1.18	0.40	Desjardin 2003
Myriophyllum	<150		0.02	Fairchild et al. 1998
Najas sp.	15	1.67		Fairchild et al. 1998
Potamogeton	63	0.69		Forney and Davis 1981
Vallisneria	141	0.40		Forney and Davis 1981

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Although not included in the compilation because they were conducted in estuarine water near 10 ppt salinity, studies on *Myriophyllum spicatum* and *Potamogeton perfoliatus* by Kemp et al. (1985) and Jones et al. (1986) are consistent with the vascular plant results in Table 1. For both these species, oxygen production-based reductions in photosynthesis (Kemp et al. 1985) indicated EC50s to be near or below 50 μg/L in the first two weeks of exposure (although some lessening of these effects was apparent in the ensuing two weeks). For *Potamogeton perfoliatus*, radiocarbon fixation-based reductions in photosynthesis (Jones et al. 1986) indicated the EC_{50} to be between 50 μg/L and 100 μg/L.

2.4 Statistical Distribution of Toxicity Relationship Parameters

The SGR EC_{50} data in Table 1 were $\log_{10}$ transformed and subject to an analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistica (Version 8.0, StatSoft, Tulsa, OK, USA). A nested ANOVA showed no significant differences between genera within the larger taxonomic groups identified in Table 1, so the analysis was simplified to a one-way ANOVA on these taxonomic groups, with each test result being treated equally regardless of the number of tests within a species or genus. This analysis indicated significant differences among the taxonomic groups, with the mean $\log_{10}(EC_{50})$ being 2.09 for green algae, 2.35 for diatoms/cryptomonads, 2.42 for blue-green algae, and 1.93 for vascular plants (Table 2). These log values correspond, respectively, to median EC_{50}s of 123, 224, 263, and 85 μg/L. Standard errors on these mean $\log_{10}(EC_{50})$s varied from 0.07 to 0.12 (Table 2), depending on the number of observations for each group. It should be further noted that the values for Chromalveolata and Cyanobacteria were based on only a few studies, underscoring the uncertainty of these inter-taxa differences. The within-group variability did not differ significantly between taxonomic groups, with the within-group standard deviation ranging from 0.29 to 0.35 and the pooled value being 0.33 (Table 2). The overall, unweighted mean and standard deviation of all $\log_{10}(EC_{50})$s were 2.12 and 0.37, this higher standard deviation being due to including the intergroup variability. Basing the analysis on genus means rather than individual tests produced similar values for the overall mean and standard deviation – 2.07 and 0.35.

The steepness parameter (Steep) data in Table 1 were also $\log_{10}$ transformed and subject to ANOVA. The ANOVAs showed no significance differences either between genera or the

Table 2. Summary statistics for SGR-based toxicity relationships from Table 1 (based on individual tests within designated taxonomic group).

Taxonomic Group	log(EC_{50})			log(Steep)		
	Mean	Std. Dev.	Std. Err. of Mean	Mean	Std. Dev.	Std. Err. of Mean
Green Algae	2.09	0.33	0.07	-0.03	0.17	0.04
Diatoms/Cryptomonads	2.35	0.29	0.12	-0.03	0.12	0.05
Blue-green Algae	2.42	0.35	0.12	-0.12	0.15	0.07
Vascular Plants	1.93	0.34	0.09	-0.07	0.23	0.06
Overall	2.12	0.37	0.06	-0.05	0.18	0.03

broader taxonomic groups. The within-group means ranged from -0.03 for the green algae and diatoms to -0.11 for the blue-green algae, with an overall mean of -0.05 (Table 2). The steepness distribution is therefore described here simply as this overall mean for $\log_{10}$(Steep) (corresponding to a median value for Steep of 0.89) and the overall observed standard deviation (0.18) (Table 2). Using genus means rather than individual observations resulted in a very similar log mean (-0.08) and standard deviation (0.16). A correlation analysis also showed no significant correlation between $\log_{10}(EC_{50})$ and $\log_{10}$(Steep), so these parameters will be treated independently in any analyses.

2.5 Uncertainty of PATI Relationships

The toxicity data analyses here provide the basis for computing an overall measure of toxic impact on an assemblage of plant species as a function of concentration. However, this does involve some issues regarding data selection and processing that will be relevant to uncertainty analyses presented in Section 4 of this document.

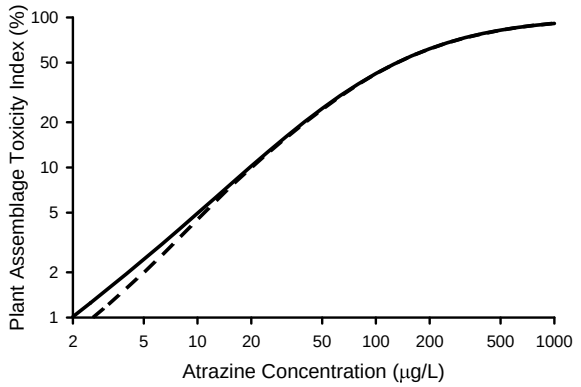
One issue is whether PATI should be calculated directly from the individual tests in Table 1 (using the overall median steepness for any test without a measured steepness) or be based on the overall distributions of $\log_{10}(EC_{50})$ and $\log_{10}$(Steep) summarized in Table 2. For the individual tests, calculating PATI is simply a matter of averaging the toxicity relationships across all the tests. For the summary distributions, calculating PATI requires multiplying the level of toxic effect expected for a particular EC_{50} and Steep by the probability density for that combination of EC_{50} and Steep, and doing this for all possible combinations of EC_{50} and Steep. Mathematically, this can be expressed as follows, where the function "*tox"* (the expected toxicity at exposure concentration C and for toxicity parameters EC_{50} and Steep) is multiplied by the function *"dens"* (the density function for the joint probability distribution of EC_{50} and Steep), and this product is then integrated across all values of EC_{50} and Steep.

$$PATI = \int \int tox(C, EC_{50}, Steep) \cdot dens(EC_{50}, Steep)\, dSteep\ \ dEC_{50} \qquad \textbf{(Equation 2)}$$

Rather than evaluating this by numerical integration, it was estimated by randomly sampling 10000 pairs of EC_{50} and Steep from the density function (assumed to be bivariate log normal with means and standard deviations as in Table 2), applying the toxic relationship function (Eq. 1) to these random pairs, and taking the mean of these toxicity values. Based on repeated tests of this process, 10000 points were sufficient to evaluate this integral with a relative error of <0.5%.

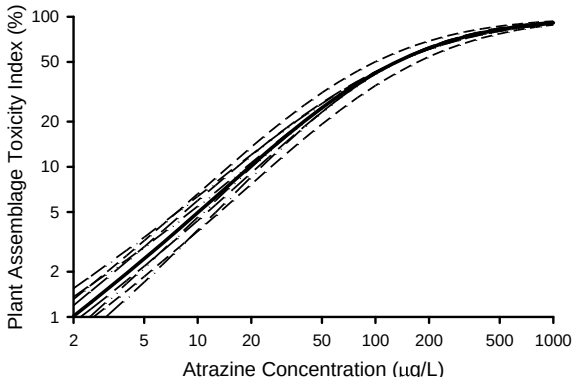
Figure 8 provides a comparison of these two calculations methods, showing a negligible difference for concentrations >10 µg/L, with the difference growing to about 30% at 2 µg/L and a PATI value of ca. 1. This calculation method issue would thus appear not to be a significant uncertainty source, but its impact on risk characterization will be examined in Section 4.

Figure 8. PATI relationships based on the toxicity relationships for individual tests (dashed line) versus based on the overall summary distribution of the relationship parameters EC50 and Steep (solid line).



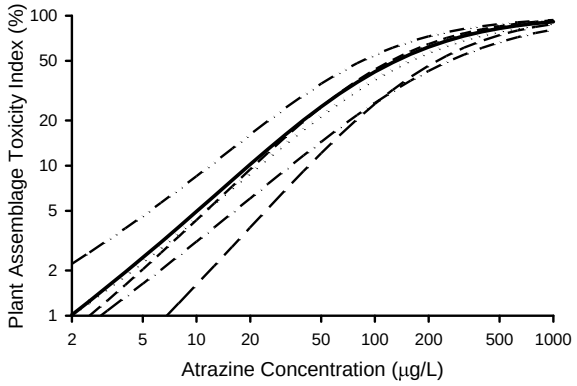
Another issue is the uncertainty associated with PATI relationships because of the finite number of toxicity relationships used in its formulation. This uncertainty is reflected in the standard errors for the means of the toxicity relationship parameters (log_{10}(EC50), log_{10}(Steep)) reported in Table 2, as well as the uncertainty in the parameter standard deviations. Figure 9 shows how PATI based on the overall distribution in Table 2 would vary by changing the mean and standard deviations of the parameters to their lower and upper 95% confidence limits. At most concentrations, the largest effects are for the uncertainties in the mean log_{10}(EC50), but the other uncertainties become substantial at lower concentrations, with the uncertainty for the mean log_{10}(Steep) approaching a factor of 2 at 2 µg/L and a PATI value of ca. 1. The impact of this uncertainty on risk characterizations will also be considered in Section 4.

Figure 9. Comparison of best estimate of overall PATI relationship (solid line), to 95% confidence limits for the mean for logEC_{50} (short dash) and logSteep (dash-dotted) and the standard deviations for logEC_{50} (long dash) and logSteep (dash-double dotted).



A third issue is that the PATI relationships in Figures 8 and 9 represent an assemblage of plant species and tests defined by the available test data, but different assemblages are possible by preferentially selecting or weighting particular taxa. Figure 10 contrasts PATI relationships based (a) on the overall distributions of $log_{10}(EC_{50})$ and log_{10}(Steep) in Table 2, (b) the separate distributions in Table 2 for the four major taxa, and (c) a composite distribution based on equal representation of the four major taxa (in contrast to the overall distribution being unweighted across all tests regardless of taxon). The PATI values for the overall distribution and the taxa composite have negligible differences, but the individual taxa can have substantial differences from the overall relationship based on their relative sensitivity, with the diatom parameters resulting in greater than 2-fold difference over a broad concentration range, reaching more than 5-fold at 2 µg/L. The effects of various options for defining the plant assemblage on risk characterization also will be examined in Section 4.

Figure 10. Comparison of PATI for the overall species composition (solid line) to green algae(short dash), diatoms(long dash), blue-green algae(dash-dotted), vascular plants(dash-double dotted), and a composite of the four taxa (dotted).

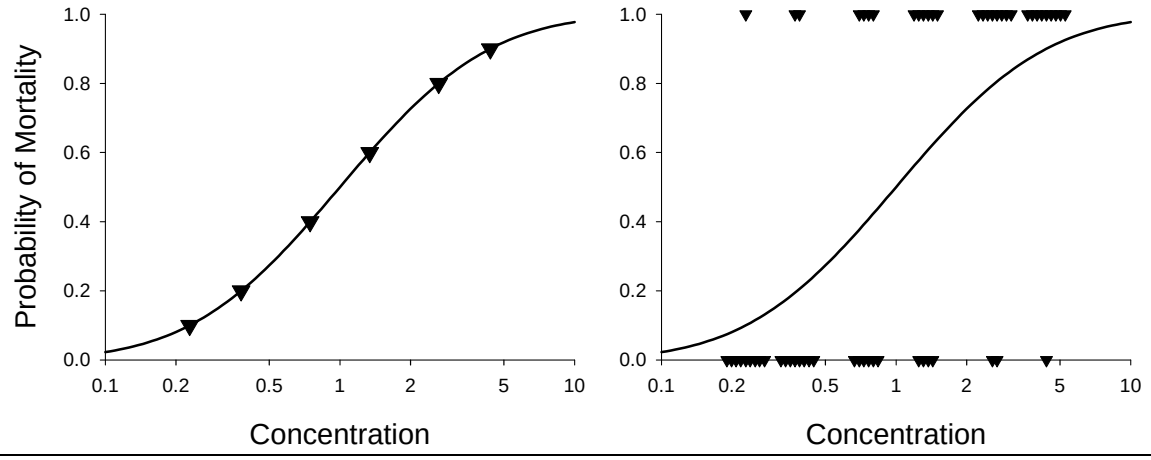


3. USING EXPERIMENTAL ECOSYSTEM DATA TO SPECIFY THE LOC FOR PATI

Using the experimental ecosystem data to determine an LOC for the cumulative PATI involves relating a binary response (yes/no effect for each experimental ecosystem treatment) to a quantitative measure for the severity of the exposure (cumulative PATI). Before presenting this process, it would be useful to first discuss a similar but more familiar analysis.

Mortality in a toxicity test also involves a binary response – an individual organism either dies or not. Mortality data is often plotted as the fraction of a group of organisms that died (at an observation time) vs. the concentration to which the group was exposed, shown in the left panel of Figure 11. However, such data can also be plotted based on the response of each individual organism (0 if alive, 1 if dead), shown on the right panel of Figure 11, in which offsets are used to show points that actually have the same concentrations. Probit analysis is a common method applied to such data to generate a sigmoidal relationship for the probability of mortality at each concentration, this relationship being the same in the left and right panels because both panels represent the same information and analysis.

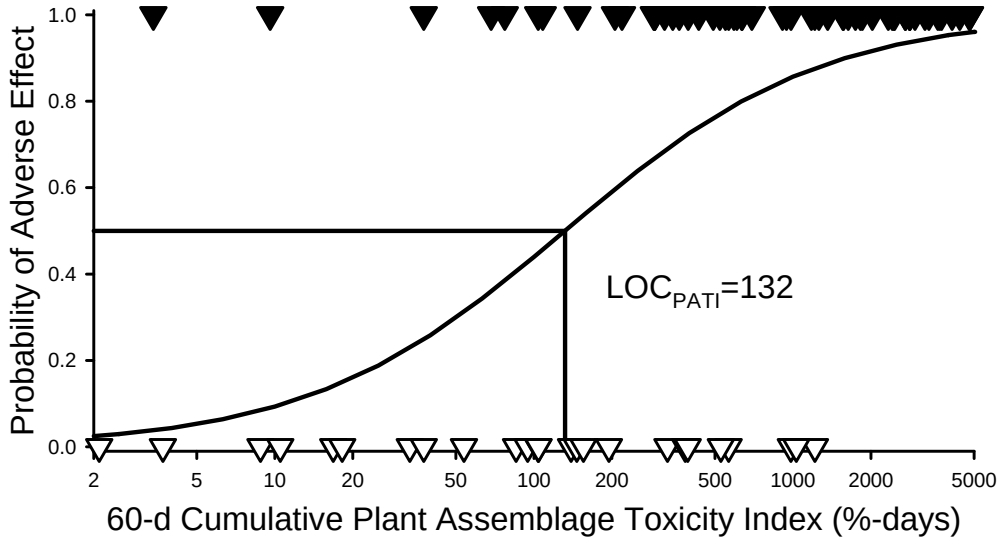
Figure 11. Probit analysis as an example of binary data analysis. For a hypothetical toxicity test, left panel shows fraction (of 10 organisms) which died at each exposure concentration while right panel plots individual organism response as 0 (if survived) and 1 (if dead). Lines denote probit relationship for probability of death.



Because probit analysis uses the binary response of the individual organism as the basic observation, it is actually more directly related to the right panel of Figure 11 than to the left. Furthermore, if individual organisms all have different exposures, the presentation format of the left panel cannot be used (i.e., there are no groups of replicate organisms upon which to compute fraction survival), but a plot such as in the right panel can still be done and probit analysis is still appropriate. For example, if the offsets for the points in the right panel of Figure 11 actually represented different concentrations, probit analysis could still be applied even without replicate points at the same concentration.

The experimental ecosystem data provide an analysis situation analogous to the survival data in the right panel of Figure 11. Figure 12 replots the experimental ecosystem data from Figure 2 as binary effects (1 if there is an effect, 0 if there isn't) vs. a $PATI_{60d}$ value. (For the purpose of this example, the overall distribution of toxicity values in Table 2 was used as the basis for PATI, along with the 60-d assessment period. The basis for these choices is discussed further in Section 4.)

Figure 12. Experimental ecosystem data plotted as effect/no effect versus $PATI_{90d}$, fitted to a logistic relationship for the probability of an effect versus PATI.



Although there is a clear increase in the probability of effects as $PATI_{60d}$ increases in Figure 12, there also is considerable overlap between effects and no effects with respect to $PATI_{60d}$, especially in the 100 to 200 range for $PATI_{60d}$. This variability/overlap issue was already noted regarding Figure 2, and should be viewed here in terms of any particular $PATI_{60d}$ value having a probability of eliciting an effect across the variety of experimental ecosystem studies used here. That there is a probability, rather than a certainty, of having an adverse effect at any $PATI_{60d}$ value is again indicative of sensitivity differences among the systems and/or various experimental uncertainties. Across all $PATI_{60d}$ values, there would be an underlying relationship for this probability, illustrated by the curve on Figure 12.

This probability relationship can be quantified using probit or similar binary analyses. Field et al. (1999, 2002) applied binary analysis to sediment toxicity assessments of a similar nature (i.e., relating binary effect data to an exposure concentration), but rather than the Gaussian distribution-based relationship of probit analysis, they applied a very similar, but simpler, probability relationship based on the logistic equation. For describing the probability of effects in the experimental ecosystem set as a function of $PATI_{60d}$, this logistic probability expression can be formulated as:

$$P = \frac{1}{1 + {PATI_{50\%} / PATI_{60d}}^{S}} = \frac{1}{1 + 10^{S \cdot \log_{10} PATI_{50\%} - \log_{10} PATI_{60d}}} \qquad \textbf{(Equation 3)}$$

where P is the probability (percent scale) of an adverse effect at a $PATI_{60d}$ value, $PATI_{50\%}$ is the $PATI_{60d}$ value at which P=0.5 (50% chance of an effect over the range of experimental ecosystems), and S is a steepness parameter (>0) for the relationship.

Although P is the underlying probability of an actual adverse effect, this equation is not appropriate for analyzing the data in Figure 12 because it does not reflect certain errors in the statistical analysis regarding whether an experimental ecosystem treatment is concluded to have

an adverse effect. Most importantly, Type I error (the probability of concluding a treatment has an effect when it actually does not) is typically set at 0.05. This means that, although the actual probability of an adverse effect approaches zero as PATI approaches zero per Equation 1, the probability of stating that there is an effect does not approach zero, but rather approaches 0.05. Type II error (the probability of concluding a treatment does not have an effect when it actually does) will also affect the curve, but it is not possible to adjust for this without detailed information on the statistical power of the various tests. However, because Type II error will go to zero as concentration increases, it will not affect the upper asymptote of the curve like Type I error affects the lower asymptote, and thus will not overtly affect the basic sigmoidal shape of the curve being fitted. The binary regression used in the LOC methodology will therefore use a logistic model with a lower asymptote of 0.05, modifying Equation 2 as follows:

$$P_{data} = \frac{1 + 0.05 \cdot {PATI_{50\%} / PATI_{60d}}^{S}}{1 + {PATI_{50\%} / PATI_{60d}}^{S}} \qquad \textbf{(Equation 4)}$$

where P_{data} refers to the probability of a data point being stated to have an effect, in contrast to P being the actual probability of having an effect.

Using Equation 4, a maximum likelihood analysis was conducted on the data in Figure 10 to generate estimates for the equation parameters, $PATI_{50\%}$ and S. Using these parameter estimates, the curve in Figure 12 was calculated, but using Equation 3 rather than Equation 4 so the curve shows the actual estimated P, not P_{data}. Once estimated, this curve provides a basis for making risk management decisions regarding what PATI value is considered an LOC. For example, for Figure 12, a risk management decision to use P=0.5 would result in an $LOC_{PATI60d}$ of 132 %-days.

This $LOC_{PATI60d}$ of 132 %-days represents substantial reductions in growth for this plant assemblage for short exposures (e.g., 44% for a three day exposure), but progressively smaller effects for longer exposures (e.g., 10% for two weeks, 5% for four weeks). However, it is important to remember that PATI is a relative index that is used here to compare experimental ecosystem results and to apply them to other exposure time-series. It is the experimental ecosystem results that define the effects that are of concern, not LOC_{PATI}.

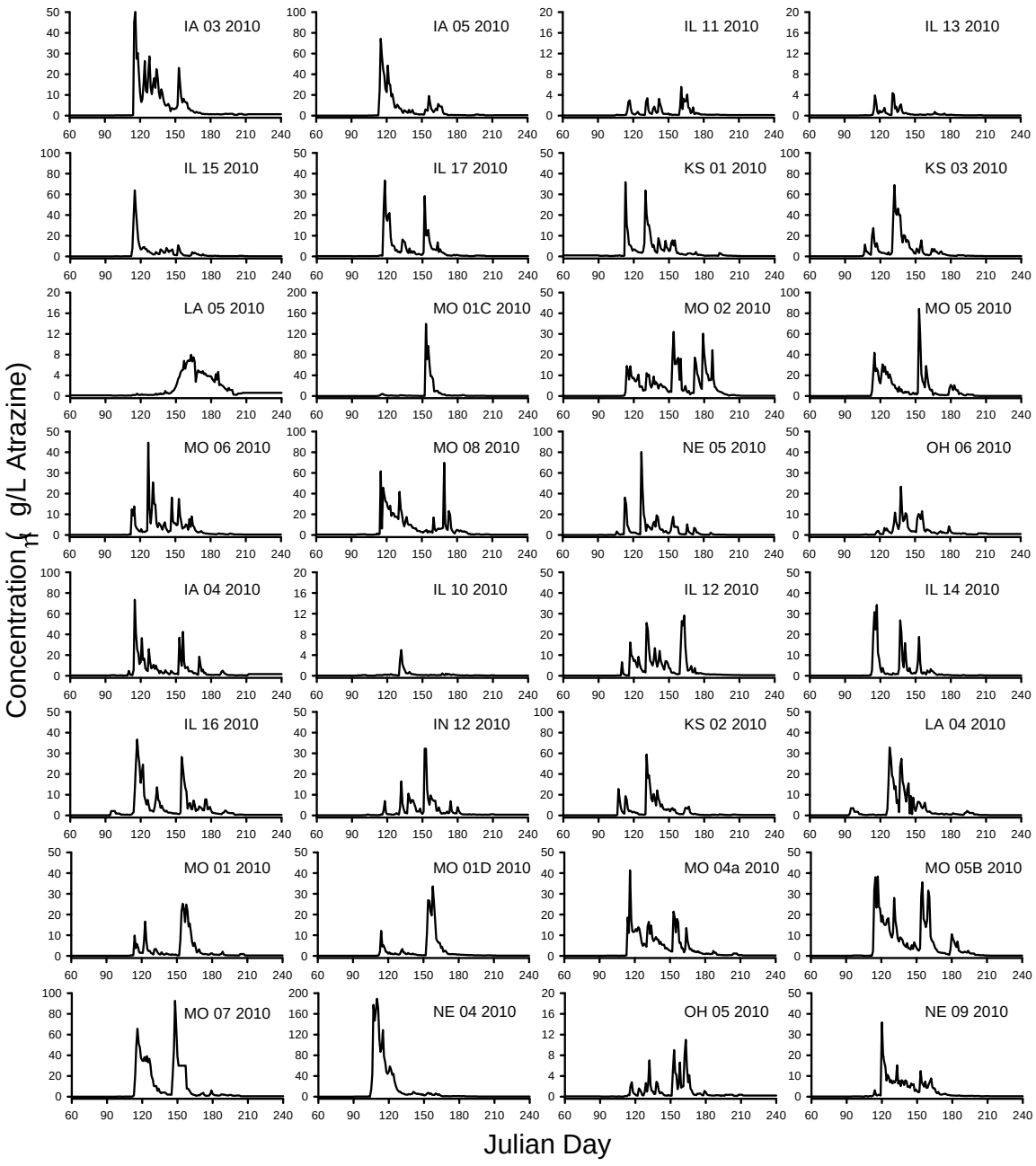
To reiterate, the level of protection is not the impact on the toxicity relationships for this specific plant assemblage as embodied in PATI. PATI is only being used to describe the ***relative*** effects of different exposure time-series, so that it is only being assumed these ***relative*** effects for this assemblage of plant toxicity relationships are relevant to the plant community effects of concern in aquatic ecosystems.

4. IMPLEMENTATION OF PATI-BASED RISK ASSESSMENT METHODOLOGY

4.1 Example Field Exposure Time-Series

Figure 1 provided three example field exposure time-series (chemographs) for use in the problem definition. In this section, method parameterization and performance evaluations will involve a larger set (Figure 13) of chemographs from the 2010 monitoring program to provide a greater diversity of exposures for evaluating the methodology.

Figure 13. Example atrazine chemographs used in methodology evaluations.



4.2 Parameterization Issues for PATI-based LOCs

Implementing a PATI-based methodology requires specifying (a) the toxicity relationships (EC_{50}s and Steeps) to use in PATI calculations and (b) the assessment period over which to evaluate cumulative PATI.

4.2.1 Assessment Period – Issues and Options

Because exposure outside the assessment period is considered inconsequential by PATI, this period needs to be long enough to encompass (a) exposures of significance to establishing LOC_{PATI} from the experimental ecosystems (Figure 2) and (b) effects expected from seasonal field exposures (Figure 13). However, it should not be any longer than necessary, in order to avoid uncertain inferences regarding (a) cumulative effects of low concentrations and (b) widely separated exposures that are independent regarding ecological effects. A 60-d assessment period would include all or almost all periods of effect in the example chemographs of Figure 13. It also encompasses the duration of all but a few of the experimental ecosystems in Figure 8. Most importantly, it is just slightly shorter than the longest treatment with no effect – being significantly shorter than this would underestimate how long exposure can be without causing effects and thus be too conservative. A shorter period would also assume that less exposure was needed to elicit some of the effects than actually was involved. That this period is longer than many experimental ecosystem exposures with effects is not inappropriate because the effects at these exposure periods would still be considered unacceptable when viewed from the perspective of this longer assessment period (e.g., if a 30-d exposure showing effects had been monitored for another 30 d with not atrazine exposure, the effects during the first 30 d would still be considered unacceptable despite any recovery that occurred during the second 30 d). The few experimental ecosystem treatments with effects at durations longer than this are of no consequence to setting the LOC_{PATI}, because they simply verify significant effects for high PATI values. To evaluate the suitability of 60 d as the assessment period and the consequences of other choices, sensitivity analyses below will address how risk characterizations would differ for assessment periods from 30-d to 120-d. A 30-d assessment period is included in this sensitivity analysis to document the impact of a period that is arguably too short, in that it is less than the duration of a substantial percentage of the experimental ecosystems treatments that discriminate effects and no effects, and inadequately covers periods of substantial exposure in the example chemographs.

4.2.2 Toxicity Parameter Distributions – Issues and Options

The review and analysis of single-species toxicity test data in Sections 2.2 and 2.3 provide the basis for specifying toxicity parameter distributions for PATI calculations, but there are options and uncertainties in applying this information, which were already discussed to some extent in Section 2.4:

(a) Should PATI calculations be directly based on the discrete estimates for the toxicity relationship parameters (EC_{50} and Steep) in Table 1, or should the methodology follow the typical assessment practice of using the data to estimate sensitivity distributions (Table 2), and basing calculations on such distributions?

(b) Should the methodology be weighted in some manner for taxonomic groups, or follow standard practice (e.g., typical SSDs) of not adjusting for the relative representation of different taxa in the available data?

(c) Should calculations be based on average results for each species or genus, or on individual tests?

The strategy here was to use a default starting point of the distributions based on all the available, individual toxicity observations (i.e., the "overall" distributions of $\log_{10}(EC_{50})$ and $\log_{10}$(Steep) summarized in Table 2). Sensitivity analyses were conducted to determine how substantially risk characterizations varied for alternatives from this default, including (a) the use of discrete parameter estimates in Table 1 instead of these default distributions (as was done for Figure 8), (b) different weightings of the major taxonomic groups (such as in Figure 10), and (c) basing distributions on genus means instead of individual test results. Based on this sensitivity analysis, decisions can be made regarding how these issues should be addressed in the final methodology.

4.3 Sensitivity Analyses for PATI-Based LOCs

4.3.1 Sensitivity Analysis for Assessment Period

Using the overall (default) toxicity parameter distributions specified in Table 2, effects assessments were made for each of the example chemographs in Figure 13, using assessment periods of 30, 60, 90, and 120 d. These assessments proceeded as follows:

(a) The daily PATI values for each experimental ecosystem exposure were calculated. As illustrated in Figure 3, this involves computing, for each daily exposure concentration, an average effect across a set of toxicity relationships. When the toxicity relationship parameters are represented by distributions, this calculation is conducted as described in Section 2.5.

(b) The daily PATI values were used to calculate cumulative PATI values for 30-, 60-, 90-, and 120-d assessment periods for each experimental ecosystem exposure. When the exposure duration exceeded the assessment period, the contiguous period of exposure resulting in the highest cumulative PATI value was used.

(c) For each assessment period, a binary logistic regression was conducted as described in Section 3.2. The LOC_{PATI} was set to the $PATI_{50\%}$ estimate from this regression (50% probability of an effect).

(d) Daily PATI values were computed for each of the example chemograph in Figure 11. Cumulative PATI values for each assessment period were calculated for the contiguous period of exposure resulting in the highest value.

(e) For each assessment period and example chemograph, risk was characterized by calculating the EEF and CEF (see Figure 3 and associated text for definition of these terms).

Figure 14. Sensitivity of risk characterization to assessment period length, based on effects exceedence factors at 20 selected sites monitored in 2010.

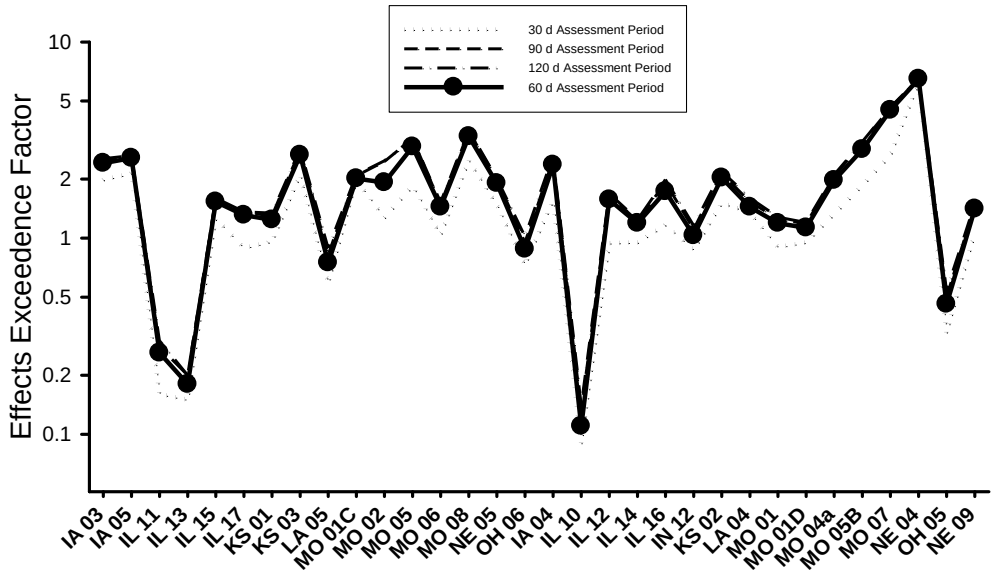


Figure 14 illustrates how the assessment period affects risk characterization, as represented by the EEF. (CEFs showed patterns very close to the EEFs and are not included here.) Relative to the proposed assessment period of 60 d, increasing the assessment period to 90 or 120 d has negligible influence (<10% except for a 25% effect for site MO 02) on the risk characterization. In contrast, using a 30-d assessment period reduces the EEF, relative to 60 d, by 4-40%, the larger reductions being associated with sites with substantial exposures for more than 30 d. Using such a short averaging period not only poorly covers experimental ecosystem exposures, but also is tantamount to asserting that the major portions of many field exposures should be ignored.

4.3.2 Sensitivity Analysis for Toxicity Information

Using the 60-d assessment period, risk characterizations were made for each of the example field exposures in Figure 13 using the following options for toxicity information:

(1) The overall distributions for logEC50 and logSteep reported in Table 2 (default).

(2) The individual logEC50 distributions for the four major taxonomic groups in Table 2 (using the overall distribution for logSteep).

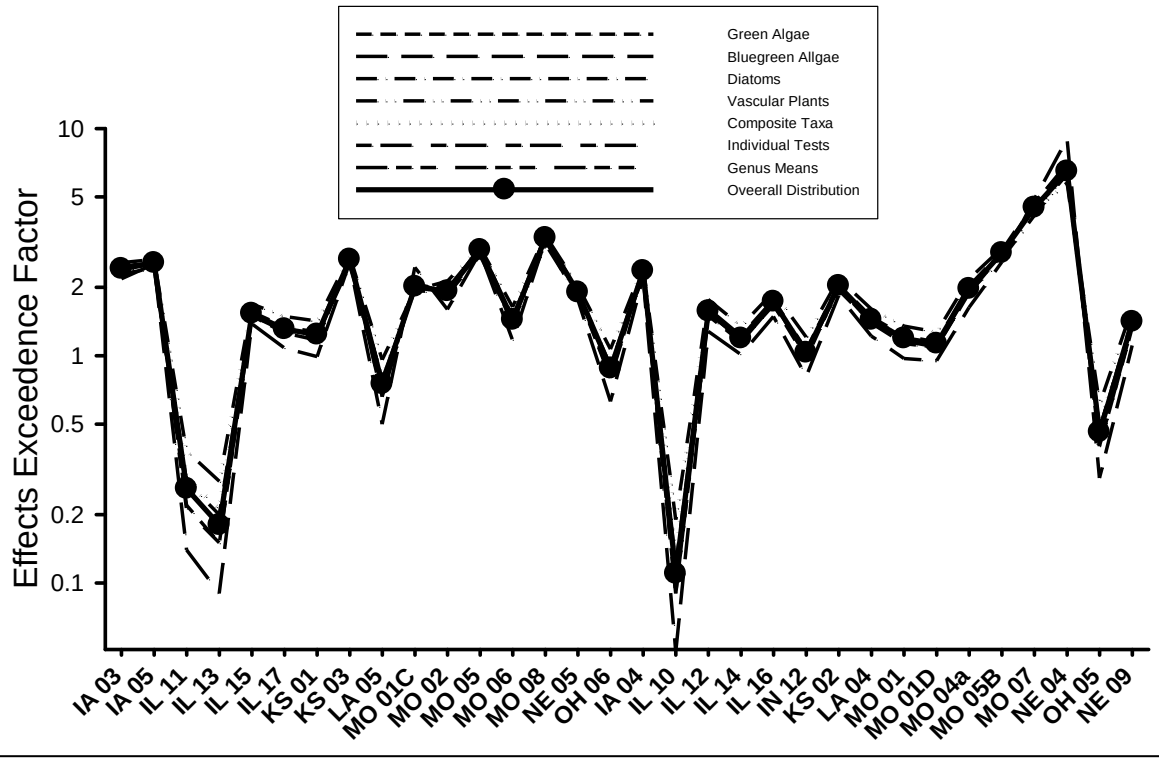
(3) An equal-weighted composite of the logEC50 distributions for the four major taxonomic groups.

(4) The individual tests in Table 1 (using the average value of -0.05 for log(Steep) for tests in which this was not determined).

(5) The overall distribution using genera means rather than individual tests (Section 2.3).

These evaluations were conducted analogously to the protocol described above for the assessment period evaluations and are summarized in Figure 15. For most options (green algae,

Figure 15. Sensitivity of risk characterization to selection of toxicity data, based on effects exceedence factors at 20 selected sites monitored in 2010



diatoms, composite taxa, genus means), the EEF deviations from the default option were generally negligible, averaging <3% and never exceeding 13%, except for the lowest EEFs (<0.3) where some deviations reached 20% but are of no consequence to risk assessment because of the low values. For the bluegreen algae option (the most tolerant group), EEFs usually are lower, but in two cases higher, than for the default option – averaging 11% lower and ranging from 37% lower to 29% higher, except for the lowest EEFs, where they are about 50% lower. For the vascular plants (the most sensitive group), EEFs usually are higher, but in two cases lower, than for the default option – averaging 10% higher and ranging from 11% lower to 33% higher, again except for the lowest EEFs, where they are 40-70% higher.

This small sensitivity of these risk characterizations to the toxicity information used in PATI might seem surprising, but this is because the experimental ecosystems, not the toxicity distributions, determine the level of concern. The toxicity distributions are only being used to assess the expected relative effects of different exposure times-series, and these relative effects are similar whether the plant assemblage is sensitive or tolerant. Using a more sensitive set of toxicity data will result in higher PATI values for both the experimental ecosystem treatments and the field exposures, so that the net effect on the EEFs is much less than that on PATI itself. There is still some effect on EEFs because PATI is not linear with concentration, so that more sensitive toxicity data will increase the relative importance of prolonged low atrazine concentrations, which are more prevalent in the field chemographs than in the experimental ecosystem exposures.

Because this sensitivity analysis shows such small effects of alternative compositions of the plant assemblage, the recommendation here is to use the overall toxicity distribution in Table 2 that was used as the default for this analysis and which is more in keeping with effects reflecting a broad assemblage of organisms.

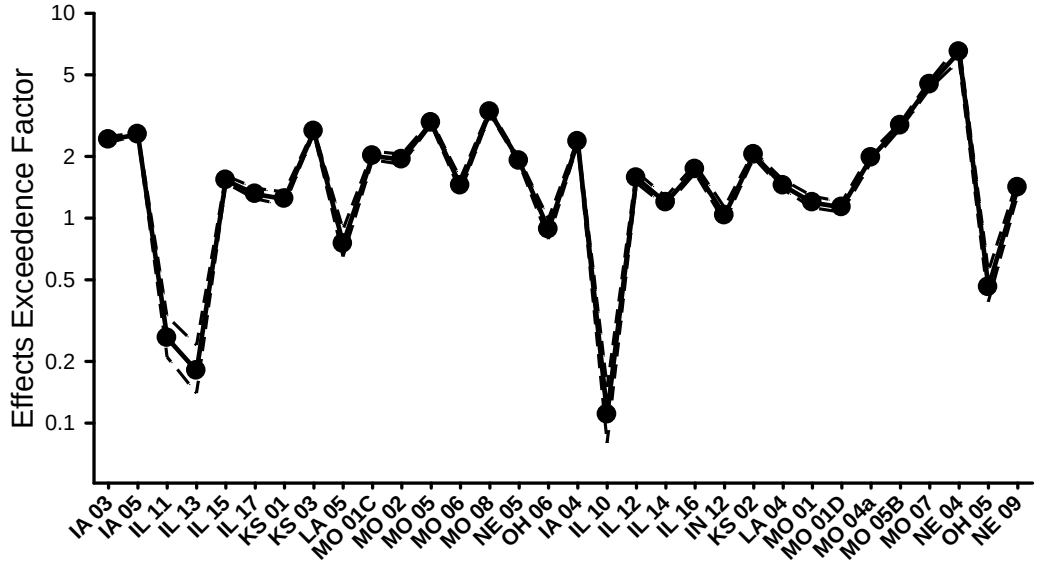
4.4 Contribution of Uncertainties in Toxicity Distributions to Assessment Uncertainty

Although varying the taxa selection in Section 4.3.2 had small effects on risk characterizations, they were not negligible and also involved substantial changes to only one parameter of four that describes the toxicity distributions – the mean of the $\log_{10}(EC_{50})$. More evaluation is needed of the total uncertainty on EEFs not just from uncertainty on the mean of the $\log_{10}(EC_{50})$ but also on the standard deviation of $\log_{10}(EC_{50})$ and the mean and standard deviation of $\log_{10}$(Steep).

To this end, an uncertainty assessment was conducted that involved (a) generating 1000 sets of toxicity parameters (means and standard deviations for both $\log_{10}(EC_{50})$ and $\log_{10}$(Steep)), (b) determining the LOC_{PATI} for each parameter set, and (c) determining the EEF for each example chemograph for these 1000 combinations of toxicity parameters and LOC_{PATI}s. The 1000 means for $\log_{10}(EC_{50})$ and $\log_{10}$(Steep) were generated by random sampling from normal distributions with the overall means and standard errors of the means for these variables reported in Table 2 (overall distribution). The 1000 standard deviations for $\log_{10}(EC_{50})$ and $\log_{10}$(Steep) were generated by random sampling from chi-square distributions based on the standard deviations of these variables reported in Table 2 (overall distribution).

Figure 16 summarizes this uncertainty analysis, comparing the 10th and 90th uncertainty percentiles to the median results. Excluding the lowest EEFs (<0.3), the lower bound for the EEF varies from 85% to 98% of the median among the chemographs, with an average of 95%, while the upper bound varies from 102% to 120% of the median, with an average of 107%.

Figure 16. Uncertainty analysis for risk characterizations due to uncertainties in toxicity distributions used to parameterize PATI. Solid line denotes EEFs based on best estimates of toxicity parameter distributions. Dashed lines denote 10th and 90th percentiles due to uncertainty of these distributions.



Although this demonstrates that uncertainties in the toxicity data used to parameterize PATI result in very little uncertainty in the final risk characterizations, this is only one component of total uncertainty for the methodology. If uncertainty estimates are to be provided, they would need to reflect all important sources[2], compared to which the uncertainties in the toxicity distributions used by PATI should be negligible.

4.5 Summary and Recommendations Regarding LOC Methodology

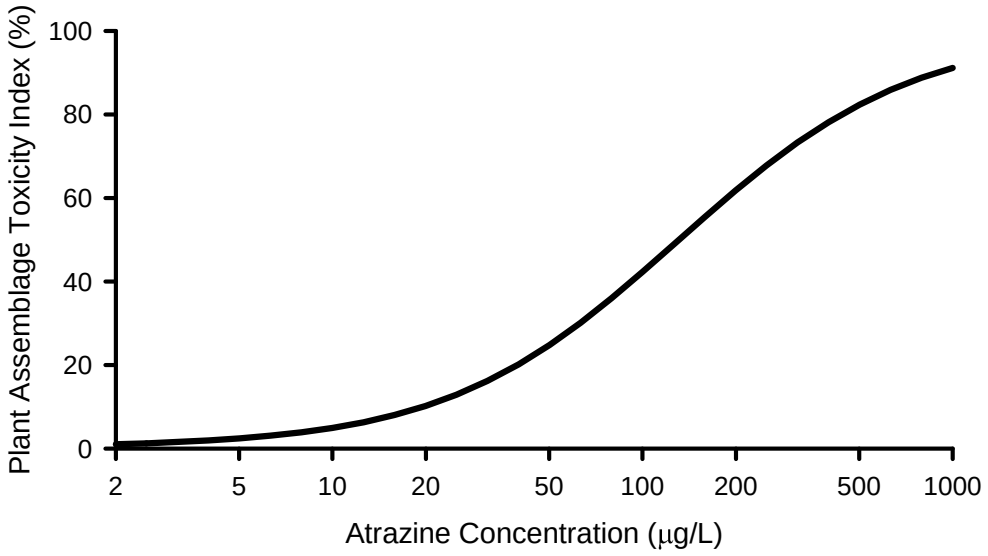
As noted in Section 1, this LOC methodology starts with experimental ecosystem studies regarding effects of atrazine on aquatic plant communities. Each experimental ecosystem treatment must be characterized as to (a) whether there is or is not an unacceptably adverse effect and (b) the atrazine concentration time series. This characterization is provided by U.S.EPA (2011) and is summarized in Appendix B. The basic problem addressed here is how to extrapolate effects among the different shapes of exposure time-series in the experimental ecosystems and from the experimental ecosystems to even more markedly different exposure time-series in natural systems. This is done with an effects index that specifies the relative toxic severity of different time-series. The recommendation here is that this index be the 60-d cumulative PATI value. This index is formulated and applied as follows:

(1) Based on available toxicity tests with individual aquatic plant species, relationships of the specific growth rate versus atrazine concentration are developed and used to specify statistical distributions for the parameters for these relationships (e.g., EC_{50}, steepness). For the toxicity test data addressed in this report, the tests were described using a logistic relationship of SGR versus log atrazine concentration, and the distributional recommendations were for the log(EC_{50}) to have a mean 2.12 and standard deviation 0.37 and for the log(Steep) to have a mean of -0.05 and a standard deviation of 0.18, based on an unweighted analysis across all tests. Although some effects of plant taxa on the toxicity relationships were indicated, alternative distributions using different weightings of the test results had only small effects on assessment results. However, the distributions recommended here should still receive additional review regarding other available data and taxonomic composition/shape.

(2) The relationship of daily PATI values to atrazine concentration should be developed for the assemblage of species described by the distributions for the toxicity relationship parameters (EC_{50} and steepness). This requires integrating the expected toxic response across the joint distribution of the parameters; this integration is best accomplished by randomly selecting a large number (e.g., 10000) of EC50/steepness pairs from these distributions, determining the toxicity relationship for each parameter pair, and averaging across all these relationships (note: because it is a numerical method for integrating across the distributions, this random selection is only done once and then applied to all subsequent PATI calculations for the specified toxicity parameter distributions). For the distributions specified here, this results in the following relationship of daily PATI values to atrazine concentration (Figure 17):

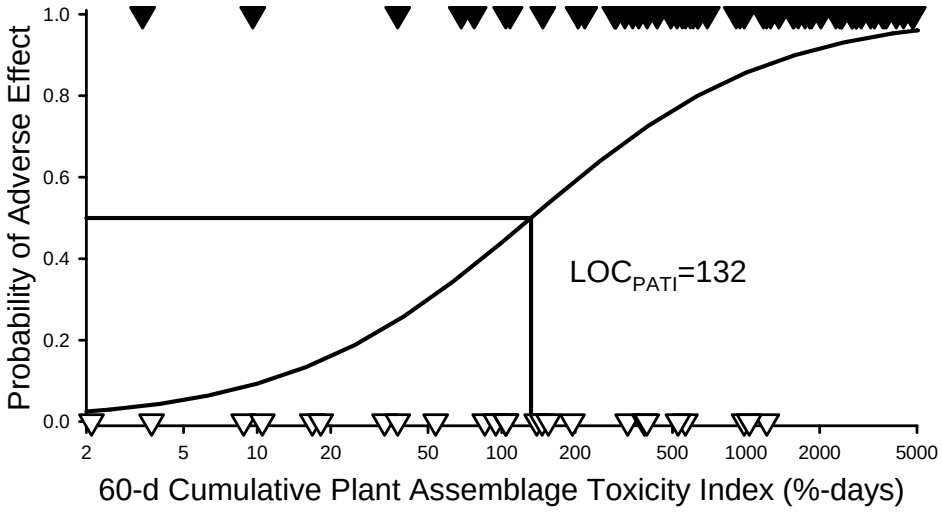
[2]An example of another source of error in the overall methodology is the uncertainty in the log(LOC_{PATI}) from the logistic regression. When the best estimates of the overall toxicity distributions are used in calculating PATI, the standard error for log(LOC_{PATI}) is 0.16 from the binary regression analysis, which produces a 10^{th} to 90^{th} percentile range for the CEF of 55-183% of the median. Other sources of uncertainty include exposure characterizations and experimental ecosystem results.

Figure 17. Relationship of PATI to atrazine concentration.



(3) Based on this relationship of daily PATI to atrazine concentration, a cumulative PATI value (=the sum of the daily PATI values) is calculated for each experimental ecosystem exposure to provide a measure for the total relative toxic impact of that exposure. This cumulative PATI value must be limited to a time frame (assessment period) consistent with risk management goals and the experimental ecosystem data. The binary effects determination for each exposure is plotted against the cumulative PATI values, an assessment period of 60 days, and a regression is performed to describe the probability of effect versus PATI. For the daily PATI relationship and the experimental ecosystem dataset used here, this results in the relationship already shown in Figure 12 and repeated in Figure 18:

Figure 18. Experimental ecosystem data plotted as effect/no effect versus $PATI_{90d}$, fitted to a logistic relationship for the probability of an effect versus PATI.



858 The above relationship describes the probability of effect versus log PATI using the logistic
859 equation with a log(EC_{50}) for PATI of 132 and a steepness of xxx. If the EC_{50} is the designated
860 level of concern, the LOC_{PATI} is 132 ug/L. These particulars are contingent on the toxicity data
861 set used for PATI, the experimental ecosystem dataset, and a risk management decision
862 regarding what probability of effect is of concern, and thus would change if any of these factors
863 is modified.

864 (4) This level of concern for PATI is applied to environmental data by calculating the cumulative
865 PATI for each environmental exposure time-series of interest. The effects exceedence factor
866 (ratio of $PATI_{60d}$s calculated for field exposures of interest to this LOC_{PATI}) (EEF) will be used
867 to determine whether the exposures exceed a level of concern. If desired, iterative calculations
868 will be used to determine the concentration exceedence factor (CEF) by which the exposure
869 exceeds a level of concern. FORTRAN-based computer programs and associated input files for
870 this implementation have been developed and are separately available from the author.

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1148

APPENDIX A

SINGLE-SPECIES PLANT TOXICITY TEST REVIEW

This appendix provides a summary for each report and journal article reviewed for developing the compilation of EC_{50}s and steepness values for the relationship of plant specific growth rate (SGR) to atrazine concentration. Bold numbers in the tables or text denote values from each study selected for inclusion in the compilation.

A.1 Protocol for Application of Toxicity Test Data

A.1.1 Acceptability of measurement variables

(1) The preferred measurement variable for assessing atrazine effects was plant biomass (dry weight, or wet weight if procedures provided consistent removal of adhering water), but measures that are approximately proportional to biomass (algal cell count or cell volume, duckweed frond count) were also accepted.

(2) If measures outlined in ***(1)*** were not available, O_2 evolution or ^{14}C fixation measurements were accepted provided that they were not significantly compromised by any lag in inducing effects and their relationship to SGR could be defined.

(3) Data based just on chlorophyll content were not used because the chlorophyll content per cell can change markedly in response to atrazine, leading to markedly different EC_{50}s for chlorophyll than for actual biomass (see discussion in Section 2.2.1 in main report text). Similarly, optical density was not accepted because it also is affected by chlorophyll content, often being measured near a chlorophyll absorbance maximum.

A.1.2 Translating reported data into SGR EC_{50} and steepness parameter values

The nature of the data and the level of detail provided in the reviewed reports/papers varied widely, requiring several different procedures for translating the reported data into the elements of the data compilation: the SGR EC_{50}, a steepness for the SGR vs. atrazine concentration relationship, and the SGR_C.

A.1.2.1 Initial and final biomasses (or surrogate) were reported for a concentration series.

The preferred data were reported initial and final biomasses (or acceptable surrogates) for all treatment concentrations, from which SGRs would then be computed. A regression analysis of SGR vs. atrazine concentration (C_{ATZ}) was then conducted, resulting in characterizing both the EC_{50} and the steepness for the relationship based on the basic measurements in the study. The analyses were by least-square, nonlinear regression using Version 1.2 of the software package TRAP (Toxicity Relationship Analysis Program) (U.S.EPA Mid-Continent Ecology Division, Duluth, MN, http://www.epa.gov/medatwrk/Prods_Pubs/trap.htm), using the "logistic equation" model option and the log-transform option for C_{ATZ}. This model option uses the logistic equation to provide a sigmoidal regression function shape, but is a regression of a continuous variable, not binary logistic analysis:

$$SGR = \frac{SGR_C}{1 + e^{4 \cdot Steep \cdot \left(\log_{10} C_{ATZ} - \log_{10} EC_{50} \right)}}$$

The defining parameters for this function are the control SGR (SGR_C), the $\log_{10}(EC_{50})$ for the SGR, and a measure of relative steepness ("Steep") defined as $\left| d(SGR/SGR_C)/d(\log_{10}(C_{ATZ})) \right|$ at the EC_{50}.

A.1.2.2 SGRs or relative SGRs were reported for a concentration series.

If the author reported SGRs (based on biomass or acceptable biomass surrogates) for all treatment concentrations, but not the actual initial and final biomasses, these SGRs were used directly in regression analysis as described in ***(A1)*** above to obtain the SGR_C, SGR EC_{50}, and steepness parameter. If the reported SGRs were relative (fraction of the control), the regression was conducted to obtain an EC_{50} and steepness to include in the compilation, but not an SGR_C, although in some cases the latter was specified separately by the author(s).

A.1.2.3 EC_{50} for the SGR was reported with or without slope.

If the author computed SGRs, but only reported an SGR-based EC_{50} without SGRs for individual treatment concentrations, the author-calculated SGR EC_{50} was included in the compilation. If the author also specified the type of relationship used in the EC_{50} estimation and a slope for that relationship, this information was converted to the steepness parameter of the relationship used in EPA's regressions; otherwise no steepness was compiled. If the author separately provided information on the SGR_C, this also was included in the compilation.

A.1.2.4 Multiple EC_ps for growth reported; SGR_C reported.

(a) If multiple EC_ps for growth over a specified duration (t) and the SGR_C for that duration were reported, SGRs corresponding to these biomass-based EC_ps were calculated using the equation:

$$SGR = \frac{1}{t} \ln \left(1 - \frac{p}{100} \cdot e^{SGR_C \cdot t} + 1 \right)$$

In other words, this is the value for the SGR at the concentration causing a p% decrease in growth. The resultant SGRs (and their associated concentrations) were then subject to regression analysis to provide estimates for the SGR EC_{50} and steepness. This provided a SGR EC_{50}, a steepness, and a SGR_C for the compilation.

(b) If the author did not specify multiple EC_ps for growth, but did provide the biomass-based EC_{50}, the type of relationship used in this EC_{50} estimation, and the slope for that relationship, additional EC_ps for growth ($p \leq 90\%$) were calculated for this author-reported curve and also converted to SGRs. These were then subject to regression analysis to provide estimates for the SGR EC_{50} and steepness, although any confidence limits on these estimates would not be valid given that the data points were not independent. Rather, this was simply a mechanism to convert the the author-reported curve for biomass-based ECs to the equivalent curve for SGR-based ECs.

(c) If the smallest SGR was more than 75% of the SGR_C for either of the above options, the regression analysis was not conducted because this would involve too much extrapolation to estimate the SGR EC_{50}. However, the possibility of extrapolating this SGR to the SGR EC50 per ***A.1.2.6*** below was then considered.

A.1.2.4 Multiple EC_ps for growth reported; SGR_C not reported.

If multiple EC_ps or an EC_{50}/slope combination for algal growth were reported, but an SGR_C was not reported, the process in ***A.1.2.4*** above was still used, but using SGR_Cs reported for other studies on test species in the same taxonomic group. Because this involves using data from other experimental systems and test species, three separate analyses were conducted using median (low-high) estimates for the SGR_C of 1.35 (1.05-1.74) for green algae, 1.03 (0.80-1.32) for diatoms, and 0.65 (0.50-0.83) for blue-green algae. The SGR EC_{50} and steepness from the regression analysis using the median SGR_C estimate were included in the compilation, provided the SGR EC_{50}s derived using the low and high SGR_C estimates differed by no more than a factor of 2.0.

[*The low/mid/high SGR_C estimates were based on ANOVA of logSGR_Cs from algal studies in which SGR_C was reported (see Table 1 in Section 2.2). Analyses using Statistica (Version 8.0, StatSoft, Tulsa, OK) provided a log mean for each major algal taxonomic group (0.135 for green algae, 0.013 for diatoms, -0.189 for blue-green algae) and a pooled standard deviation (0.122). The low/mid/high estimates for SGR_C were based on calculating the mean ± 1 std.dev. of these log values and then taking antilogarithms. Separate SGR_C values for species within a taxonomic group were not justified because of large within-species variability relative to between-species variability, as evidenced in Table 1 and other sources (e.g., Saenz et al. 1997).*]

A.1.2.6 EC_{50} only for growth reported; SGR_C reported.

If the EC_{50}s for growth over a specified duration (t) and the SGR_C for that duration were reported, this biomass-based EC_{50} was equated to an SGR EC_p using the following equation to determine p:

$$SGR = \frac{1}{t}\ln\ 0.5\cdot\ e^{SGR_C\cdot t} + 1$$
$$p = 1 - \frac{SGR}{SGR_C}$$

When only the SGR_C and this single SGR are available, no regression analysis is possible. Rather, this SGR EC_p was extrapolated to an SGR EC_{50} using the equation $EC_{50} = EC_P \cdot 10^{\sqrt{2\cdot p}/S}$, where S is based on regression curve steepnesses from other studies. Because this involves using data from other experimental systems and test species, three estimates of the SGR EC_{50} were made using low, middle, and high estimates for the steepness of 0.68, 0.95, and 1.31. The estimate for the SGR EC_{50} from the middle steepness estimate was included in the compilation, but only if the estimates based on low and high steepness differed by less than a factor of 2. This factor of 2 requirement was met if p>16 for the estimated SGR EC_p.

[*An ANOVA of all the log steepness determined in all studies indicated no significant differences among species or broader taxonomic groups, so the overall mean and standard deviation of the log steepnesses were used to set low/mid/high estimates.*]

A.1.2.7 EC_{50} only for growth reported; SGR_C not reported.

When only an EC_{50} for growth was reported and a study-specific SGR_C was not reported, the biomass-based EC_{50} was equated to SGR-based EC_ps per section ***A.1.2.5*** using low, middle, and high estimates for SGR_C. Then, each of these SGR-based EC_p estimates was extrapolated to SGR EC_{50} estimates per section ***A.1.2.6*** using low, middle, and high steepness estimates. The SGR EC_{50} estimate based on the middle SGR_C and steepness estimates was included in the compilation, provided the extremes of the estimates varied by less than a factor of 2. This factor of 2 requirement resulted in this procedure being applicable for green algae tests of up to 2 d long, but tests could be up to 4-d long for blue-green algae and up to 3-d long for diatoms. Extrapolating EC_{50}s for biomass to SGR EC_{50}s were just too uncertain for tests longer than this.

A.1.2.8 Oxygen evolution or ^{14}C fixation reported

(a) If the exposure and measurement periods were short enough so that biomass did not change appreciably during these periods, and if initial biomasses were either measured or could be treated as approximately the same among treatments, oxygen evolution and radiocarbon fixation rates were treated as proportional to SGR and EC_ps for these rates were treated as comparable to SGR-based EC_ps. However, this also required consideration of whether these periods were so short that any lag in the induction of toxicity would significantly perturb the measurement. Hersh and Crumpton (1989) and Millie and Hersh (1987) reported effects on oxygen evolution that were >50% within several minutes of exposure to atrazine concentrations that caused similar effects on biomass-based SGRs. Thus, data were accepted provided an induction lag of 5 min would not significantly confound results.

(b) When the exposure and measurement periods were the same and biomass changed enough over the period to substantially affect estimated EC_ps, oxygen evolution and radiocarbon fixation were treated as being proportional to net growth ($e^{SGR \cdot t}$), and ECs were converted to an SGR basis analogously to procedures described above for biomass-based ECs.

(c) If a substantial exposure period of duration "t" preceded a short measurement period, so that the treatments would start with significantly different initial biomasses for the oxygen evolution/radiocarbon fixation measurement period, these measures were treated as being proportional to $SGR \cdot e^{SGR \cdot t}$; i.e., the biomass accretion in the exposure period prior to the start of measurement is $e^{SGR \cdot t}$ and the oxygen evolution/radiocarbon fixation rate is proportional to the SGR times that biomass accretion. This required converting ECs to an SGR-basis using approaches analogous to that described above for biomass.

A.1.3 Issues regarding biomass surrogates and variability.

One uncertainty issue occurred when the biomass surrogate was cell counts made manually using a hemocytometer or similar device. In some cases, cell density estimates were based on <100 cells counted in total for the control treatment and just several cells for atrazine treatments with

large effects. Even 100 cells represents about +/-20% uncertainty in the cell density. Therefore, it was desired to have >200 cells counted in the control treatment in order to have reasonable discrimination between the control and treatments with 25-50% reduced growth. Another area of concern was frond counts for duckweed, and how closely such counts mirror biomass when growth is limited and thus might have a greater percentage of newer, small fronds. Where possible, it was desired to have at least a 4-fold increase in the number of control fronds so that the counts were not excessively dominated by new, small fronds. A final area of concern was macrophyte shoot tests at times when controls had not increased by at least 50%, especially if this was measured by shoot length, which can change disproportionately to shoot weight when photosynthesis is inhibited. No firm rules were imposed with regard to any of these concerns, because any uncertainty depends on the number of replicates in a test, the specific times, the variability among replicates, etc. How these concerns were evaluated are identified in the summaries for each study in Appendix A.

A.1.4 Treatment of data at multiple times

When biomasses or biomass surrogates were reported at multiple times within a test duration, analyses were conducted for each time; however, the compilation selected results from only one of these times. This time was long enough to avoid problems with uncertain measurements of biomass early in some tests (e.g., the hemocytometer count issue discussed above), but short enough to avoid potential biases associated with declining SGR_C discussed earlier. Again, no firm rules could be adopted for this because of various study-specific factors and because it involved balancing uncertainties at early times with those at later times. The decision process regarding this are provided in the summaries for each study in Appendix A.

A.2 Review Summaries

A.2.1 Algae

(1) Gala and Giesy 1990

The authors conducted a 96-h flask test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, enumerating cell density based on hemocytometer cell counts. Concentrations were measured. Illumination was continuous at 40 $\mu E/m^2/s$, temperature was 24 C. They reported average SGRs over 96 h at each treatment concentration, which were directly used in EPA regression analyses. Data for earlier times were not reported, but authors noted the use of extra nutrients to maintain exponential growth. Due to the duration and growth rates, cell densities would have been high enough to avoid concerns about low numbers of individuals manually counted.

Measured (Target) Concentration (µg/L)	Author Measured SGR (1/d)
Control	**1.007**
64 (60)	0.773
121 (120)	0.508
261 (250)	0.244
499 (500)	0.013

EC_{50} (µg/L)	**125 (80-194)**
Steepness	**1.07 (0.46-1.77)**

(2) van der Heever and Grobbelaar 1996

The authors conducted a 72-h flask test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, determining biomass (dry weight), cell density (electronic particle counter), and chlorophyll (by both spectrometry and fluorometry) at 0, 24, 48, and 72 h. Concentrations were nominal. Illumination was continuous at 300 µE/m^2/s and temperature was 23 C. The authors graphically reported ***relative*** (to control) SGRs based on all these measures. Author-reported ECs based on chlorophyll were substantially (almost 3X) higher than for cell density and biomass, and were not used in accordance with the review guidelines. Relative SGRs for cell density and biomass were estimated from the figures, reported in the table below, and used in EPA regression analyses to determine EC_{50} and steepness. The results based on dry weight were selected for use because EC_{50}s were modestly higher for cell density (average LC_{50} = 406 by cell density, 311 by weight) indicative of decreases in mass per cell at higher atrazine concentrations, so that using cell density would slightly reduce the apparent sensitivity of biomass to atrazine. The results at 1 d were selected for use because it was unknown whether control growth rates declined with time, given that only relative SGRs were reported, and because use of an electronic particle counter should have avoided the problems with low manual cell counts at early times.

Nominal	Author Relative SGR, Cell Counts			Author Relative SGR, Dry Weight		
Conc (µg/L)	1d	2d	3d	1d	2d	3d
1	1.13	1.30	1.22	1.06	1.10	1.00
5	0.98	1.00	0.95	1.00	1.18	1.02
10	0.98	1.11	1.07	0.84	1.02	0.91
50	0.97	0.97	0.97	0.88	1.00	0.93
100	0.95	1.10	1.08	0.83	1.06	0.91
500	0.35	0.30	0.30	0.18	0.30	0.33
1000	0.37	0.34	0.37	0.10	0.10	0.10
5000	0.20	0.12	0.10	0.00	0.00	0.00
EC_{50} (µg/L)	439	370	401	**236 (149-376)**	352	352
Steepness	0.56	0.79	0.78	**1.01 (0.52-1.50)**	1.44	1.14

(3) van der Heever and Grobbelaar 1997

The authors conducted a 30-min oxygen evolution assay for ***Selenastrum capricornutum*** exposed to multiple atrazine concentrations. Concentrations were nominal. Illumination was continuous at 300 µE/m^2/s and temperature was 23 C. Oxygen evolution rates relative to the control were reported graphically and the values in the table below were estimated from the

figure. Because of negative responses at high concentrations, the regression in this review included a non-zero asymptote at high concentrations, but the EC_{50} is still defined relative to zero oxygen evolution, not this negative asymptote, so that this would best reflect net production. Although there was no prior exposure before oxygen evolution measurements were made, the measurement period was long enough relative to the 5-min induction standard that these results were accepted. It should be noted that the results are consistent with those for a flask test by the same authors discussed above.

Nominal Conc (μg/L)	Author Relative Oxygen Evolution
5	100
50	84
500	27
1000	0
5000	-14
10000	-25
EC_{50} (μg/L)	**223 (144-346)**
Steepness	**0.61 (0.42-0.80)**

(4) Kallqvist and Romstad 1994

The authors conducted a 72-h flask test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, enumerating cell density using an electronic particle counter. Concentrations were nominal. Illumination was continuous at 70 $\mu E/m^2/s$ and temperature was not reported but followed OECD standards of 23±2 C. The authors conducted a regression analysis of probit-transformed ***relative*** SGRs, reporting an SGR EC_{50} of **110 μg/L (95% cl = 99-121)** and an EC_{10} of 27 μg/L. Individual SGRs were not reported, but these two ECs allow estimating a steepness of **0.90** for the sigmoidal function used in this review.

The authors also conducted 3- to 6-d microplate exposures of several algal species to atrazine. The duration of the test varied with species in order to be within the period of exponential growth. Illumination was continuous at 70 $\mu E/m^2/s$ for green algae and 30 $\mu E/m^2/s$ for others. For these exposures, relative SGRs for each treatment were reported graphically. Values estimated from the figures are provided in the following table, along with EC_{50}s and steepnesses estimated from regression analysis of this data. The EC_{50} for *Selenastrum* was higher for the microplate exposures than for the flask tests (although by less than 2-fold), suggesting that the microplate exposure methodology might involve factors that lead to decreased apparent sensitivity (e.g., nutrient or atrazine reductions, although the former would not be expected if exponential growth was maintained). These microplate-based numbers were still compiled for use in subsequent analyses because the *Selenastrum* EC_{50}s was well within the reported range of results for this species from other studies; however, this possible source of uncertainty was recognized in applications of these data.

Nominal Concentration	Relative SGR (% of Control)					
	Selenastrum capricornutum	*Chlamydomonas noctigama*	*Cyclotella sp.*	*Cryptomonas pyrinoidifera*	*Microcystis aeruginosa*	*Synechococcus leopoliensis*
0	100	100	100	100	100	100
3.2				95	110	
10	100	100		99	102	91
20			100			
32	93	97		99	95	80
60			100			70
100	73	84	96	91	88	57
200			95	85		
320	34	53	61	69	69	30
600			40		58	16
1000	12	28	17		33	13
2000				5		
3200	0	7	0	0	3	0
6000						
10000		0			0	0
EC_{50}	**201 (177-227)**	**378 (313-456)**	**462 (383-556)**	**494 (415-587)**	**603 (443-820)**	**136 (116-159)**
Steepness	**0.79 (0.68-0.90)**	**0.65 (0.53-0.77)**	**1.22 (0.80-1.64)**	**1.15 (0.85-1.45)**	**0.77 (0.43-1.11)**	**0.59 (0.52-0.66)**

(5) Hoberg 1991a

The author conducted a 96-h flask test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, enumerating cell density based on hemocytometer cell counts. The author provided a data table of cell counts at 1, 2, 3, 4 d at multiple concentrations; initial cell counts were reported to be $1 \cdot 10^4$. Concentrations were measured and were stable for 4 d (concentrations were 2X higher than target due to diluting error). Light was continuous at 450-500 ft-c and temperature was 24-25 C. SGRs were calculated by EPA for each duration and concentration and used in regression analyses to estimate EC_{50} and steepness. Substantial and continuing declines in control SGRs were observed, so that the growth rate over 2 d was 24% less than that over the first day. However, cell counts over the first day were lower than desired for good quantification and the drop in SGR could be partly due to uncertainty in both the initial and day 1 cell counts. Therefore, day 2 values were selected for the data compilation.

Conc (µg/L)		Author Cell Counts ($/10^4$)				Calculated SGR (1/d)			
Target	Measured	1d	2d	3d`	4d	1d	2d	3d	4d
0	-	10.0	33.0	71.7	105.0	2.30	**1.75**	1.42	1.16
32	76	5.0	9.3	49.7	101.7	1.61	1.12	1.30	1.16
63	130	2.3	5.0	31.7	27.7	0.83	0.80	1.15	0.83
120	250	1.7	4.0	1.7	2.0	0.53	0.69	0.18	0.17
240	510	0.7	2.3	2.0	1.0	<0.00	0.42	0.23	0.00

490	970	0	0	0	0	-	-	-	-
EC_{50} (μg/L)						109	**131 (59-290)**	180	161
Steepness						1.13	**0.62 (0.18-1.10)**	2.61	2.42

(6) Hoberg 1993a

The author conducted a 96-h flask test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, enumerating cell density based on hemocytometer cell counts. The author provided a data table of cell counts at 1, 2, 3, 4 d at multiple concentrations; initial cell counts were reported to be $0.3 \cdot 10^4$. Concentrations were measured and were stable for 4 d. Light was continuous at 300-450 ft-c and temperature was 24 C. SGRs were calculated by EPA for each duration and concentration from these counts. The control SGR during the first day was exceptionally high (3.32/d) and dropped to more typical levels during subsequent days. In addition, SGRs were high during the first day even at the highest atrazine concentration (2.30/d at 450 μg/L), and also dropped to more typical values during subsequent days (<0.1/d). These atypical results might represent an error in the initial cell density, the reported value of which was atypically low and could not be verified. These data were therefore not used.

(7) Caux et al. 1996

The authors conducted a 4-d microplate test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, enumerating cell density using an electronic particle counter. Light was continuous at 60 $\mu E/m^2/s$ and temperature was 24 C. The authors only provided a 4-d EC_{50} for cell density (26 μg/L), with no data on actual cell counts at test termination for atrazine treatments. No information was provided on actual treatment concentrations. However, they did report an initial cell density of $1 \cdot 10^4$ and a final control cell density of $1\text{-}2 \cdot 10^6$, corresponding to an SGR_C of 1.15-1.32/d, a relatively narrow range. Based on the midrange of the reported final control cell counts, an SGR_C of 1.25/d was used for adjusting the cell density-based EC_{50} to the SGR (1.08/d) that would result in half the final control density. The authors also reported a probit slope of 4.95 for the cell density vs. $\log_{10}C$ relationship, which allowed calculation of other EC_ps for cell density (e.g., EC_{16} and EC_{84} corresponding to ±1 standard deviation in probit equation) and their corresponding SGRs. Per item ***A.1.2.4(b)*** in the protocol, these estimated SGRs were subject to regression analysis to estimate the SGR EC_{50} and steepness. Confidence limits are not reported because this regression was not based on independent data points, but on a conversion of the reported relationship for the cell density ECs.

p (% reduction in cell counts)	ECp (μg/L)	4-d Cell Density (10^4 cell/ml)	Estimated SGR (1/d)
0		1.50	1.25
16	16.4	1.26	1.21
50	26	0.75	1.08
84	41	0.24	0.795
EC_{50} (μg/L)			**50**

Steepness			**1.66**

(8) Versteeg 1990

The author compared three assays of atrazine effects on *Selenastrum capricornutum* growth: a 4-d flask test enumerating cell density based on hemocytometer cell counts, 5-min ^{14}C fixation after 30-min exposure, and 30-min oxygen evolution. Light was continuous at 86 μE/m^2/s for the flask test, 350 μE/m^2/s for the ^{14}C fixation, and 250 μE/m^2/s for the oxygen evolution; temperature was 24 C. Reported EC_{50}s were 50 μg/L for 4-d cell density, 100 μg/L for ^{14}C fixation, and 380 μg/L for oxygen evolution. Data for individual treatments were not reported for atrazine, but were for simazine, another triazine herbicide. Measurement variables (cell densities, ^{14}C fixation rate, oxygen evolution rate) relative to the control are provided in the following table for simazine. Simazine showed differences among the EC_{50}s based on cell densities, ^{14}C fixation rate, and oxygen evolution similar to atrazine. SGRs based on cell density effects were also estimated per item ***A.1.2.5*** of the protocol, resulting in an SGR-based EC_{50} similar to that for ^{14}C fixation. This simazine analysis also resulted in a slope for SGR-based ECs that was included in the compilation.

Analysis of Versteeg 1990 Results for Simazine				
Concentration (μg/L)	Cell Density (% of Control)	SGR (% of Control)	^{14}C Fixation Rate (% of Control)	Oxygen Evolution (% of Control)
0	100	100	100	100
25			104	
50	78	95	103	
100	47	86		
150	23	73		93
175			59	
200	10	58		
225				80
300			38	70
500				43
EC_{50} (μg/L)	95	180	215	437
Steepness	1.58	**1.50**	1.19	1.26

Based on the experimental procedures and the results for both atrazine and simazine, this study was applied as follows regarding EC_{50}s:
(a) Because the oxygen evolution assay involved purging oxygen, with uncertain effects on photosynthesis rates and sensitivity to atrazine, these data were not used.
(b) Because the ^{14}C fixation assay included prior exposure, the results will be used. Because of the short exposure and measurement periods, the EC_{50} (**100 μg/L**) for ^{14}C fixation will be treated as being equivalent to those for SGRs.
(c) The smaller EC_{50} for the flask test cell density is likely due to it being for cumulative growth over 4 d. Per item ***A.1.2.7*** in the protocol, this had too long a duration to extrapolate the cell density-based EC_{50} to an SGR-based EC_{50} given the range of estimates for the unknown SGR_C

and steepness. However, if the steepness for simazine was used, the procedure would result in the estimates for the SGR EC_{50} from 95-115 μg/L, consistent with that for ^{14}C fixation.

(9) Larsen et al. 1986

The authors reported EC_{50}s for ^{14}C fixation rates of several algal species, measured over 2 h after 24 h prior exposure to atrazine. Light was continuous at 400 ft-c and temperature was 24 C. Because the 24-h prior exposure would result in substantially different biomasses among treatments, this measure is not proportional to the SGR, and because fixation was not cumulative over the entire period (26 h), it is also not proportional to net growth. Assuming that SGR is approximately constant within each treatment, the biomass at 24 h would be e^{SGR} and the carbon fixation over the 2-h measurement period would be proportional to $SGR \cdot e^{SGR}$, ignoring the small amount of growth over that 2 h and assuming that the measured fixation over the 2 h is approximately proportional to the SGR. Given this relationship, per item ***A.1.2.7*** of the protocol, an EC_{50} for the SGR can still be calculated from this information, if an SGR_C and steepness can be estimated for use in the following calculations:

(a) Solve for SGR_p (p = percent reduction in SGR relative to control) corresponding to the EC_{50} for ^{14}C fixation using the equation $SGR_P e^{SGR_P} = 0.5 \cdot SGR_C e^{SGR_C}$ (i.e., this equation describes what the SGR would have to be so that the function $SGR \cdot e^{SGR}$ is at half of its control value).

(b) Calculate p as $100 \cdot (1 - SGR_p/SGR_C)$.

(c) Use the estimated steepness for the toxicity relationship to extrapolate the known SGR EC_p (=EC_{50} for ^{14}C fixation) to the SGR EC_{50}.

For *Selenastrum capricornutum*, the authors reported EC_{50}s for ^{14}C fixation of 34-53 μg/L (three tests, average 43). Using this average EC_{50}, the procedure described above was conducted multiple times using the low, middle, and high estimates for SGR_C and steepness identified in the protocol for this review. The range of the resultant SGR EC_{50}s was 66-114 μg/L, narrow enough to include the median SGR EC_{50} (78 μg/L) in the data compilation. For the other species, the following table summarizes comparable calculations. For green algae, the same ratio (1.88) between the carbon fixation and SGR EC50s was used as for *Selenastrum*. For blue-green algae, the ratio used was 1.43 based on the estimates for SGR_C for blue-green algae specified in the review guidelines.

Test Species	^{14}C EC_{50} (μg/L)	SGR EC_{50} (μg/L)
Selenastrum capricornutum	43	**78**
Ankistrodesmus sp.	66	**119**
Chlamydomonas reinhardi	37	**67**
Scenedesmus obliquus	48	**87**
Chlorella vulgaris	308	**557**
Stigeoclonium tenue	175	**317**

Ulothrix subconstricta	88	**159**
Anabaena cylindrica	204	**286**

(10) Mayer et al. 1998

The authors provided an EC_{10}, EC_{50}, and EC_{90} for SGRs from a standard ISO 8692 toxicity flask test (3 d) with *Selenastrum capricornutum*. The actual temperature and light intensity was not reported, but the cited test protocol specified 60-120 μE/m2/s and 23±2 C. The author-reported SGR EC_{50} of **164** μg/L will be used, but the multiple ECs can also be used to estimate the steepness parameter for the sigmoidal relationship used in this review. The author also reported information on effects of light, temperature, pH, and nitrogen source on both control growth and toxic effects. This information indicated the SGR_C for this study under standard conditions was about **1.8/d**, but insufficient information was available to use other toxicity information for the present analysis. This study did document a 10-fold increase in chlorophyll content per cell due to atrazine exposure (200 μg/L), which provides some of the basis for not accepting this as a surrogate for biomass.

p (% reduction in control SGR)	ECp (μg/L)	Relative SGR
0		1.0
10	17.2	0.90
50	**164**	0.50
90	688	0.10
EC_{50} (μg/L)		164
Steepness		**0.79**

(11) Roberts et al. 1990

The authors conducted a 7-d flask test of *Selenastrum capricornutum* growth at multiple atrazine concentrations, enumerating cell density based on hemocytometer cell counts. Concentrations were nominal. Light was continuous at 2300 ft-c and temperature was 24 C. The authors reported the number for the doublings (cell count basis) over 3 d. This number of doublings was converted to a factor increase, which was converted to an SGR and subject to regression analysis.

Nominal Concentration (μg/L)	Number of Doublings	Relative Growth (Factor increase)	Calculated SGR (1/d)
0	7.13	140	**1.65**
50	6.64	100	1.53
100	5.08	33.8	1.17
150	4.10	17.2	0.95
EC_{50} (μg/L)			**163**
Steepness			**1.22**

(12) Parrish, 1978

The author conducted 5-d flask tests of *Selenastrum capricornutum* and *Microcystis aeruginosa* growth at multiple atrazine concentrations, enumerating cell density based on hemocytometer cell counts. Concentrations were nominal. Light was continuous at 400 ft-c and temperature was 24 C. The author provided a data table of cell counts at 3 and 5 d at multiple concentrations; initial cell counts were $2 \cdot 10^4$ for *Selenastrum* and $5 \cdot 10^4$ for *Microcystis*. SGRs were calculated from the counts for each duration and concentration. Results for *Selenastrum* are in the following table. Because there was not a substantial decline in the SGR_C and results agreed between the two durations, the 5-d results were selected for use.

Conc (µg/L) (nominal)	Author Cell Counts ($/10^4$)		Calculated SGR (1/d)	
	3d	5d	3d	5d
0	55.8	249.6	1.110	0.965
32	50.6	207.3	1.077	0.928
54	34.5	130.3	0.949	0.835
90	14.6	28.2	0.663	0.529
150	8.9	8.9	0.498	0.300
250	0.7	0.7	<0	<0
EC_{50}			115	**101 (79-130)**
Steepness			1.47	**1.61 (0.67-2.55)**

Results for *Microcystis* are in the following table. Control growth actually increased later in the test and EC_{50}s were similar for both durations, so the 5-d results were selected for use.

Conc (µg/L) (nominal)	Author Cell Counts ($/10^4$)		Calculated SGR (1/d)	
	3d	5d	3d	5d
0	14.3	77.1	0.350	0.547
65	13.2	71.6	0.324	0.532
108	12.9	26.1	0.316	0.330
180	6.5	21.5	0.087	0.292
300	5.1	9.6	0.007	0.130
500	4.7	4.0	0.000	0.000
EC_{50}			154	**164 (95-285)**
Steepness			4.2	**1.25 (0.24-2.46)**

(13) Turbak et al. 1986

The authors reported an EC_{50} of **70 μg/L** based on a 30-min oxygen evolution assay with *Selenastrum capricornutum*, with no additional information to determine the steepness of the relationship. The actual temperature and light intensity was not reported, but the test protocol specified 400 ft-c and 24 C. The methods description did indicate that there was some exposure prior to oxygen measurements, and 30 min is long enough not to be greatly perturbed by induction lags of several minutes. Therefore, this EC_{50} based on rate of oxygen evolution was accepted as informative of an SGR EC_{50}. They also reported a 59 μg/L SGR EC_{50} based on a 2-3 week bottle test. Because of the length of this test and the lack of specifics regarding it, this EC_{50} was not used, but this result does not contradict the EC_{50} based on oxygen evolution.

(14) Radetski et al. 1995

The authors reported a 72-h EC_{50} of 118 μg/L for *Selenastrum capricornutum* based on cell counts (Coulter counter) in a semistatic microplate well test. The actual temperature and light intensity was not reported, but the cited test protocol specified 60-120 μE/m2/s and 23±2 C. They also reported an initial cell count of $2 \cdot 10^4$ and a final control cell count of $6.6 \cdot 10^6$, corresponding to an SGR_C of **1.93/d**. At the reported EC_{50}, the final cell count would thus have been $3.3 \cdot 10^6$, equivalent to an SGR of 1.70, corresponding to a 12% reduction from the control value (i.e., the growth EC_{50} is an SGR EC_{12}). Per protocol item ***A.1.2.6***, this is too long of an extrapolation to estimate an SGR EC_{50} given the uncertainty in the steepness of the relationship, so an SGR EC_{50} was not computed. However, the SGR_C was used in the compilation.

(15) Abou-Waly et al. 1991

The authors conducted 7-d flask tests of *Selenastrum capricornutum* and *Anabaena flos-aquae aeruginosa* growth at multiple atrazine concentrations, measuring weights and chlorophyll concentrations. Concentrations were nominal. The authors reported SGRs for multiple durations and concentrations, but only for chlorophyll measurements. Therefore, these data were not used in accordance with item ***(A3)*** of the protocol. Reported chlorophyll-based growth rates and EC_{50}s had complex relationships to time and exposure concentration, thereby substantiating concerns about using chlorophyll measurements. For *Anabaena*, transferring organisms to control media after the end of the exposure test showed rapid recovery of growth rates.

(16) Hughes et al. 1988, Hughes 1986

The authors conducted 5-d flask tests of the growth of two algal species, *Anabaena flos-aquae* and *Navicula pelliculosa*, at multiple atrazine concentrations, enumerating cell density by electronic particle counting. Concentrations were not measured. Light was continuous, and light intensity/temperatures were 200 ft-c/24 C for Anabaena and 400 ft-c/20 C for Navicula. The author provided data tables of algal cell densities at 3 and 5 d. SGRs were calculated for each duration and concentration from these counts, based on the reported initial algal cell densities of $2 \cdot 10^4$ cells/ml.

The following table provides results for *Anabaena flos-aquae*. Because no significant effects of duration are evident on either control growth rates or the EC_{50}, the 5-d results were selected for further use.

Conc (µg/L) (nominal)		Author Cell Counts ($/10^4$)		Calculated SGR (1/d)	
		3d	5d	3d	5d
0		23.4	88.0	0.82	**0.76**
100		16.9	68.4	0.71	0.71
200		16.1	47.5	0.69	0.63
400		8.4	24.7	0.48	0.50
800		6.7	10.2	0.40	0.33
1600		3.9	5.6	0.22	0.21
3200		4.5	5.5	0.27	0.20
EC_{50}				736	**706 (440-1131)**
Steepness				0.48	**0.59 (0.35-0.83)**

The following table provides results for *Navicula pellculosa*. Because control growth was maintained or even increased through 5 d, the 5-d results were selected for further use.

Conc (µg/L) (nominal)		Author Cell Counts ($/10^4$)		Calculated SGR (1/d)	
		3d	5d	3d	5d
0		26.2	347	0.86	**1.03**
100		9.4	132	0.53	0.84
200		6.0	29.3	0.37	0.54
400		3.6	7.7	0.20	0.27
800		2.3	2.8	0.05	0.07
1600		1.9	1.9	0.00	0.00
3200		2.1	1.8		
EC_{50}				153	**217 (189-248)**
Steepness				0.80	**1.08 (0.87-1.29)**

(17) Fairchild et al. 1994, 1998

The authors assessed the effects of four herbicides on plant growth using 4-d tests with six algal species. Concentrations were not measured in exposure chambers, but the stock concentrations were verified. Because chlorophyll was used to quantify algal biomass, these data were not used here per item ***(A3)*** of the protocol.

(18) Fairchild et al. 1995, 1997

The authors conducted 4-d tests of *Selenastrum capricornutum* at multiple atrazine concentrations (as well as 15 other herbicides). Concentrations were not measured. Because

chlorophyll was used to quantify *Selenastrum* biomass, these data were not used here per item ***(A3)*** of the protocol.

(19) Burrell et al. 1985

The authors conducted an 11-d flask tests of the growth of *Chlorella vulgaris* and *Ankistrodesmus braunii* at multiple atrazine concentrations, enumerating cell density based on optical density and hemocytometer cell counts. Concentrations were not measured. Illumination was continuous at 30 $\mu E/m^2/s$ and temperature was 24 C. Initial cell densities were $1 \cdot 10^5$ and exponential cell growth was reported to be maintained for the test duration, culminating in a final cell density of $1.7 \cdot 10^6$ (SGR_C=0.26/d) in the *Chlorella* test and $3.8 \cdot 10^6$ (SGR_C=0.33/d) in the *Ankistrodesmus* test. The authors graphically reported the percent reduction in the final cell density at each atrazine concentration, which were estimated from the figure and reported in the table below. Based on the final cell densities in the control and the test durations, these percent reductions in cell density were converted to SGRs at each atrazine concentration and subject to regression analyses to determine the SGR EC_{50} and steepness. Although this test was longer than would typically be used for this compilation, the SGR_C were low enough (at least in part due to low light intensities) that total cell densities were not so high as to confound results or to doubt the authors' statement that exponential growth was maintained. However, because these SGR_Cs were so low they were not used for estimating SGR_Cs for other studies.

Ankistrodesmus			***Chlorella***		
Nominal Atrazine Conc (µg/L)	% Reduction in Growth	SGR (1/d)	Nominal Atrazine Conc (µg/L)	% Reduction in Growth	SGR (1/d)
Control	0	.331	Control	0	.258
40	19	.312	10	27	.229
60	49	.269	30	55	.185
70	66	.232	50	67	.157
100	81	.180	70	72	.142
			100	75	.131
EC_{50} (µg/L)		**104 (83-131)**	EC_{50} (µg/L)		**91 (70-118)**
Steepness		**1.41 (0.56-2.36)**	Steepness		**0.47 (0.32-0.63)**

(20) Kirby and Sheahan 1994

The authors conducted a 4-d flask test of the growth of *Scenedesmus subspicatus* at multiple atrazine concentrations; concentrations were measured. Illumination was continuous at 3500 lux and temperature was 25 C. The authors only reported EC_{50}s based on final biomass, without any information on specific treatments, growth rates, etc. Initial cell density was $1 \cdot 10^4$ cell/ml and growth was quantified by spectrophotometric absorbance calibrated to cell density. The EC_{50} based on final cell density was 21 µg/L. Because only an EC_{50} was reported and an SGR_C was not reported, estimation of the SGR EC_{50} would be per item ***A.1.2.7*** of the protocol, but this was

not done because the extrapolation would be too great (the extrapolated value would be 80 μg/L with a range of 50 to 150 μg/L). In addition, this study used optical density near the chlorophyll a maximum, and so would not be used per the review guidelines.

(21) Millie and Hersh 1987

The authors determined oxygen evolution rates in an electrode chamber for three geographical races of *Cyclotella meneghiana* exposed to different atrazine concentrations (unmeasured). Illumination was at 300 $\mu E/m^2/s$ and temperature was 25 C. The authors graphically reported the percent inhibition of oxygen evolution rate relative to controls at each concentration, and these percentages were determined from the graph and subject to regression analysis to determine oxygen evolution EC_{50} and steepness. Because these were based on a short-term (1 min) oxygen evolution and because there was prior exposure to each atrazine concentration of several minutes before oxygen evolution was measured, ECs from these oxygen evolution rates were accepted as being comparable to SGR ECs.

Nominal Atrazine Conc (μg/L)	Oxygen Evolution Rate - % of Control		
	Minnesota Race	Arizona Race	Iowa Race
1		94	92
6		95	85
31		80	77
64	89	58	62
95	78	51	54
143	71	39	40
213	53	31	34
277	40	25	21
338	32	15	22
EC_{50} (μg/L)	**225 (202-251)**	**100 (86-116)**	**114 (93-141)**
Steepness	**1.00 (0.79-1.20)**	**0.67 (0.56-0.79**	**0.65 (0.49-0.81)**

(22) Hersh and Crumpton 1989

The authors determined oxygen evolution rates in an electrode chamber of a commercial strain of *Chlamydomonas reinhardii* and of three isolates of *Chlorella* sp. obtained from an uncontaminated natural system exposed to different atrazine concentrations (unmeasured). Illumination was at 300 $\mu E/m^2/s$ and temperature was 25 C. Only the EC_{50} for the reduction in oxygen evolution rates relative to control were reported (no data on actual oxygen evolution vs. concentration), but because these were based on a short-term (1 min) oxygen evolution and because there was prior exposure to each atrazine concentration of several minutes before oxygen evolution was measured, these oxygen evolution EC_{50}s were accepted as being comparable to SGR EC_{50}s. For *Chlamydomonas*, the EC50 was **45 μg/L** and for *Chlorella* it averaged **37 μg/L** across the three isolates (range=36-41).

(23) Stratton 1981, 1984

The author measured ^{14}C fixation over 3 h and cell growth rate (by optical density) over 12-14 d for five algal species exposed to various atrazine and atrazine metabolite concentrations. Concentrations were unmeasured. For the ^{14}C fixation tests, light intensity was 7000 lux and temperature was 20 C; these were not specified for the growth test, but presumably were the same because these were also the culture conditions. For the growth tests, data other than EC_{50}s at the end of the test were not provided, except for *A. inaequalis*, and this showed non-exponential growth throughout the last 10 d of the test and indicated the EC50 was lower at 4-5 d than later in the text, although the plotted data were insufficient to quantify this. In addition, optical density was measured at wavelengths with substantial chlorophyll absorption for at least three of the species. For these reasons, the ECs from the long growth test were not used, and only the ^{14}C fixation EC50s were compiled:

	Anabaena inaequalis	Anabaena cylindrica	Anabaena variabilis	Chlorella pyrenoidosa	Scenedesmus quadricauda
^{14}C fixation EC_{50} (µg/L)	**280**	**470**	**70**	**480**	**300**

(24) Schafer et al. 1994

The authors conducted a 10-d test of the growth of *Chlamydomonas reinhardi* in a flow-through apparatus that maintained exponential cell growth, and reported EC_{50}s and EC_{10}s for growth at 4, 7, and 10 d. Concentrations were measured. The light intensity was 7000 lux with a 14/10 photoperiod and the temperature was 24 C. Information was also provided to allow estimation of the SGR_C to be 1.06/d, but no additional information on actual or relative cell counts at different concentrations and times, etc. was given. These ECs were reported to be for growth (not growth rate) and to be derived per OECD method 201, so presumably were based on "area under the curve" (AUC). They thus do not represent the difference between the biomass at the stated time and the biomass at test start, but rather the sum of these differences across the whole time interval (and thus a measure of the average increase). Because this system maintained an exponential growth and because the SGR_C is known, the EC_{50}s can be used to estimate SGRs for those concentrations, as summarized in the following table. The magnitudes of these estimated effects on the SGR are insufficient to support a regression analysis to estimate the SGR EC_{50} and steepness (due to the large extrapolation from 16% effect to 50% effect). However, per item ***A.1.2.6*** in the protocol, this SGR EC_{16} of 51 µg/L can be extrapolated to an estimate of **141 µg/L** for the SGR EC50.

Concentration (µg/L)	Duration (d) for which concentration is AUC EC_{50}	SGR (1/d)
Control	N/A	1.060
10.2	10	0.99
21	7	0.96
51	4	0.89

The authors also conducted 3-d flask tests of the growth of *Chlamydomonas reinhardii* and *Scenedesumus subspicatus* at different atrazine concentrations, measuring cell densities at 1, 2, and 3 d with an electronic particle counter. Illumination was continuous at 8000 lux and the temperature was 20 C. The authors reported 3-d EC_{50}s and EC_{10}s from these tests, but without any other effect information (e.g., actual or relative cell counts at different concentrations and times, growth rates). Because of high initial cell densities ($2 \cdot 10^5$ cell/ml) that would have led to growth-inhibiting densities based on the SGR_C from the flow-through test, the growth EC_{50} for *Chlamydomonas* (350 μg/L) cannot be converted to information on an SGR EC. For *Scenedesmus*, initial cell densities were low enough ($5 \cdot 10^4$ cell/ml) to make converting the growth EC_{50} (72 μg/L) reasonable; however, this would follow item ***A.1.2.7*** of the protocol, and the duration of the test is too long for this extrapolation given uncertainties in both SGR_C and steepness.

(25) Faust et al. 1993

The authors conducted 1-d tests of *Chlorella fusca* growth at multiple atrazine concentrations. This was a synchronized culture of 1 generation per day, in which a cell grows during the light period (14 h) and releases a set of daughter cells in the subsequent dark period (10 h); cell counts were by Coulter counter. The SGR_C for cell number would be ln(# of daughter cells) for the control treatment, but this number was not reported. This number can be as low as 4 (SGR_C=1.4/d), but in a related paper by Altenburger et al. (1990), a value of 12 was indicated (SGR_C=2.5/d). The authors reported a probit equation for cell reproduction over 24 h. The points on this probit equation corresponding to -2, -1, 0, 1, and 2 probit units from the median were calculated to provide EC_ps for cell "reproduction" (table below). Then, two sets of SGR estimates corresponding to these EC_ps were calculated based on the two alternatives for the SGR_C, and regression analyses were conducted on each of these sets of SGRs. The resultant SGR EC50 estimates did not differ markedly (table below), so the average of these were included in the data compilation.

Concentration (μg/L)	Percent of Control Reproduction	SGR (1/d)	
Control	100	1.4	2.4
2.45	97.5	1.381	2.377
6.1	84	1.272	2.243
15.1	50	0.927	1.794
37.2	16	0.398	0.957
92	2.5	0.074	0.224
EC_{50} (μg/L)		**22**	**29**
Steepness		**1.08**	**1.06**

(26) Geyer et al. 1985

The authors conducted 4-d flask tests of *Scenedesmus subspicatus* growth at multiple atrazine concentrations. The AUC EC_{50} was reported to be 110 μg/L, but other information (effects at

higher concentrations, control SGR) were not reported. This test does not meet the protocols stated earlier for extrapolating such an EC_{50} to one for the SGR.

(27) Zagorc-Koncan 1996

The author determined the net production of oxygen over 24 h (by liberated gas via Warburg-type apparatus) and increased biomass as measured by chlorophyll over 72 h of *Scenedesmus subspicatus* exposed to multiple atrazine concentrations. Light was continuous at 800 lux and temperature was 20 C. As noted in the protocol, chlorophyll is not an acceptable surrogate for biomass. Regarding oxygen evolution, the authors reported an EC50 of 25 μg/L, but because of the lengthy incubation this should be proportional to net biomass gain and not directly related to effects on SGR. To convert to an SGR-basis requires estimating SGRs based on the oxygen production and assumptions regarding SGR_C. Such estimates based on the range of SGR_C for green algae observed in other studies are included in the table below and subject to regression analysis. Variation in the assumed SGR_C did not cause great variation in the estimated SGR EC50; because of the low temperature and light intensity, the compilation used the value from the lowest SGR_C value.

Nominal Atrazine Conc (μg/L)	Estimated SGR (1/d)		
	SGR_C=1.05	SGR_C=1.35	SGR_C=1.74
Control	1.050	1.350	1.740
0.1	1.038	1.336	1.724
1.0	1.004	1.297	1.681
5.0	0.926	1.208	1.580
10	0.896	1.173	1.54
50	0.431	0.604	0.86
EC_{50} (μg/L)	**39 (27-56)**	44	51
Steepness	**0.73 (0.45-1.01)**	0.72	0.70

(28) Tang et al. 1997

The authors conducted 28 d tests with several algal species. Growth was measured based on chlorophyll measurements and optical density near the chlorophyll a maximum. Due to both the length and the type of measurement, these data were not used.

(29) Gramlich and Frans 1964

The authors conducted a 5-d flask test with *Chlorella pyrenoidosa* at several atrazine concentrations. Because biomass was measured by optical density and because initial values for biomass were not given, useful results for the compilation could not be obtained from this study.

(30) Stratton and Giles 1990

The authors examined the effect of volume and initial cell density on the toxicity of atrazine to *Chlorella pyrenoidosa*, measured by radiocarbon uptake over 24 h. Although these experiments demonstrated inhibition relative to the control and did include some treatments with approximately 50% inhibition, only one concentration was tested, absolute fixation rates were not tested, and a variety of processes might be affecting the observed inhibition. This precluded applying these data to the data compilation of interest here.

(31) Boger and Schlue 1976

The authors evaluated photosynthesis based on oxygen evolution rate after several days of exposure to atrazine and the recovery of photosynthesis upon transfer of exposed algae to clean medium and control algae to contaminated medium. However, only one concentration was tested and results could not be related to the effect concentrations desired in this review.

(32) University of Mississippi 1991

The authors evaluated growth of *Selenastrum capricornutum* (4 d) at multiple atrazine concentrations. This test involved methodological and performance problems that precluded its use, especially for determining SGR-based ECs. Chlorophyll measurements were made, but were erratic in addition to being not accepted in the protocol used here. Both cell densities and weights were also measured, but no initial cell density was specified, final densities were based on inadequate numbers of cells, and many of the measurements of final weight were negative. Atrazine effects were evident at 100 μg/L, but the next lower and higher concentration was 10-fold different (10 and 1000 μg/L) , precluding any good characterization of dose-response.

A.2.2 Vascular plants

(1) Hughes et al. 1988, Hughes 1986

The authors conducted a 5-d test with the duckweed, *Lemna gibba*, at multiple atrazine concentrations, assessing growth by frond count. Concentrations were not measured. Light was at 500 ft-c and temperature was 25 C. The authors provided data tables of duckweed frond counts at 3 and 5 d. SGRs were calculated for each duration and concentration from these counts, based on an initial frond count of 16. The following table summarizes observations and the estimated SGRs. Because control growth was less than a factor of two at 3 d, the 5-d results were selected for further use.

Conc (μg/L) (nominal)		Average Frond Counts		SGR (1/d)	
		3d	5d	3d	5d
0		29.0	49.3	0.198	0.225
100		27.0	40.0	0.174	0.183
200		19.7	29.7	0.069	0.124

400		16.3	21.7	0.006	0.061
800		16.0	16.3	0.000	0.004
1600		1.9	1.9		
3200		2.1	1.8		
EC_{50}				169	**224 (151-332)**
Steepness				2.17	**1.14 (0.43-1.85)**

(2) Hoberg 2007

The author conducted growth tests with isolated shoots of *Elodea canadensis* at multiple atrazine concentrations and at zero, dim (500 lux), and optimal (6000 lux) light levels (only the higher light level is appropriate for this review). Concentrations were measured and temperature was 20-25C. Data tables were provided for individual shoot lengths at 0 and 14 d and individual shoot dry weights at 14 d for multiple concentrations. Only dry weight is considered here (shoot lengths were a poor surrogate for growth because substantial shoot elongation was observed in low light and at high atrazine concentrations were no growth in weight was observed). This requires having an estimate of the initial dry weight, which the author reported for a separate initial sample of shoots as being 0.1346 g/shoot. It was assumed that this weight applied to the average initial shoot length (8.3 cm/shoot) so that the initial weight per cm 0.0162 g/cm. This factor was used to estimate the initial weights for each replicate tanks based on the initial shoot lengths within that tank, allowing SGRs to be computed for each tank. The following table lists the reported final weights, the estimated initial weights, and the resultant shoot weight SGRs, along with the EC50 and steepness parameter estimated by regression analysis. This regression analysis is relatively uncertain because the lowest treatment concentration corresponds to an EC68, leaving an absence of data at low to moderate effect. However, the estimated steepness is similar to others reported for this species (Table XX) so the EC50 estimate was still deemed acceptable for us.

Measured Concentration (µg/L)	Estimated Initial Average Shoot Weight (g dwt)	Reported Final Average Shoot Weight (g dwt)	Shoot Weight SGR (1/d)
0	0.133,0.120,0.129,0.121	0.420,0.415,0.420,0.471	0.082,0.089,0.084,0.097
464	0.126,0.131,0.141,0.129	0.166,0.218,0.225,0.178	0.020,0.036,0.034,0.023
853	0.137,0.139,0.136,0.153	0.213,0.179,0.184,0.185	0.031,0.018,0.022,0.009
1761	0.131,0.133,0.149,0.136	0.128,0.166,0.214,0.126	-0.001,0.016,0.026,-0.005
Regression EC_{50} (µg/L)			**204 (59-600)**
Regression Steepness			**0.52 (0.15-0.98)**

(3) Hoberg 1991b

The author conducted a 7-d test of *Lemna gibba* growth at multiple atrazine concentrations; concentrations were measured. Light was continuous and temperature was 24 C. The author provided a data table of frond counts at 3, 6, and 7 d at multiple concentrations; initial frond counts were 15. SGRs were calculated for each duration and concentration from the counts and regression analyses were conducted on these SGRs. Because of the absence of growth on day 7, the 6-d values were compiled.

Measured Atrazine Concentration (µg/L)		Average Frond Counts			SGR (1/d)		
		3d	6d	7d	3d	6d	7d
0		34.0	78.0	80.7	0.273	0.275	0.240
15		32.0	84.0	85.3	0.253	0.287	0.248
28		31.0	78.0	77.0	0.242	0.275	0.234
57		33.0	68.0	68.3	0.263	0.252	0.217
120		28.3	52.0	51.3	0.212	0.207	0.176
220		21.7	34.0	31.3	0.123	0.136	0.105
390		19.0	19.7	19.3	0.079	0.045	0.036
EC_{50}					230	**202 (174-234)**	189
Steepness					1.14	**1.24 (0.85-1.62)**	1.24

(4) Hoberg 1993b

The author conducted a 14-d test of *Lemna gibba* growth at multiple atrazine concentrations. Concentrations were measured. Light was at 400 ft-c and temperature at 24 C. The author provided a data table of frond counts at 3, 6, 9, 12, and 14 d and dry weight at 14 d. Initial frond counts were 15. Initial dry weight was unreported but it was assumed for this analysis that the initial dry weight per frond was equal to that in the control at the end (=110 mg/529=0.208 mg/frond), so that the initial dry weight would be 3.12 mg. SGRs were calculated for each duration and concentration from the counts and dry weights and regression analyses were conducted on these SGRs. Based on frond count, some reduction in control growth rate occurred after 9 d, but did not appreciably affect estimated SGR EC_{50}s. For the 14-d data, dry weights resulted in an EC_{50} 29% lower than that based on frond count. This is likely attributable to the lower dry weight/frond at higher atrazine concentrations (i.e., smaller fronds due to atrazine effects), but also could be contributed to by overestimation of the initial dry weight if control fronds at the end were on average larger than those at the beginning. This illustrates a possible weakness in the use of frond counts for duckweed tests, but also a weakness in most tests regarding measuring initial weights. Due to it being a direct measure of biomass rather than an indicator, the dry weight-based results were compiled.

Measured Atrazine Concen.	Average Frond Count					Avg dwt (mg)	Frond Count SGR (1/d)					Dwt SGR
	3d	6d	9d	12d	14d	14d	3d	6d	9d	12d	14d	14d
0	37.0	99.0	255	424	529	110	.301	.314	.315	.278	.254	.254
3.4	35.3	91.0	244	426	440	96	.285	.300	.310	.279	.241	.245

7.2	36.0	89.0	253	475	470	117	.292	.297	.313	.288	.246	.259
17	36.3	76.0	202	334	364	77	,295	.270	.289	.259	.228	.229
47	32.3	71.7	163	303	310	17	.256	.261	.265	.250	.216	.222
92	26.7	45.0	79	117	117	16	.192	.183	.185	.171	.147	.116
240	20.7	25.7	35	36	43	5	.107	.090	.094	.073	.075	.036
EC_{50}							156	133	130	129	134	**93 (72-120)**
Steepness							0.87	0.85	0.85	1.09	0.90	**1.33 (.58-2.07)**

(5) Hoberg 1993c

The author conducted a 14-d test of *Lemna gibba* growth at multiple atrazine concentrations. Concentrations were measured. Light was continuous at 450-500 ft-c and temperature was 25 C. The author provided a data table of frond counts at 3, 6, 9, 12, and 14 d and dry weight at 14 d. Initial frond counts were 15. Initial dry weight was unreported but it was assumed for this analysis that the initial dry weight per frond was equal to that in the control at the end, resulting in a estimated initial dry weight of 3.7 mg. SGRs were calculated for each duration and concentration from the counts and dry weights and regression analyses were conducted on these SGRs. As for Hoberg 1993b, dry weight-based SGRs showed a lower EC_{50} and higher steepness than frond count-basis, and were selected for the compilation.

Measured Atrazine Concen	Average Frond Count					Avg dwt (mg)	Frond Count SGR (1/d)					Dwt SGR
	3d	6d	9d	12d	14d	14d	3d	6d	9d	12d	14d	14d
0	37.2	88.7	191	277	356	88	0.303	0.296	0.283	0.243	0.226	0.226
0.53	37.3	84.7	187	257	364	82	0.304	0.288	0.280	0.237	0.228	0.221
1.3	37.0	85.7	185	241	327	94	0.301	0.290	0.278	0.231	0.220	0.231
3.0	36.7	89.7	178	284	298	90	0.298	0.298	0.275	0.245	0.214	0.228
8.3	34.3	83.3	162	278	321	72	0.276	0.286	0.264	0.243	0.219	0.212
18	32.3	71.0	136	204	258	58	0.255	0.259	0.245	0.218	0.203	0.197
44	26.0	46.3	81	132	147	24	0.183	0.188	0.187	0.181	0.163	0.134
100	20.3	26.7	35	48	53	4.2	0.101	0.096	0.094	0.097	0.090	0.009
EC_{50}							61	63	67	82	81	**49 (42-58)**
Steepness							0.78	0.95	0.91	0.099	0.96	**1.71 (.82-2.60)**

(6) Desjardin et al., 2003

The authors conducted tests on *Lemna gibba* growth at multiple atrazine concentrations and for multiple durations (1-14 d) followed by examination of recovery. Concentrations were measured. Temperature was 24-25 C and light intensity 4250-5750 lux. Rapid recovery was demonstrated, but the analyses here are concerned with effects during the exposure period.

Furthermore, this analysis will be restricted to a 7-d test, because both the longer tests (9-14 d) produced less than a 20% reduction in the SGR and the 1-3 d tests provided uncertain results due to the short duration and limited concentration range. The authors provided data at day 2, 4, and 7 d and dry weight at 7 d at multiple concentrations. Initial frond counts were 15 at day -1 and were 20-21 at the start of exposure (this 1 d period of growth was done to identify/discard chambers that showed little or no growth; despite this precaution, one control replicate had poor enough growth to be excluded as an outlier). The initial dry weight was estimated to be 2.8 mg based on the average dry weight/frond in the no-effect concentrations at the end of the exposure. SGRs were calculated for each duration and concentration from the counts and dry weights.

Measured Atrazine Concen	Average Frond Count			Avg dwt (mg)	Frond Count SGR (1/d)			Dwt SGR
	2d	4d	7d	7d	2d	4d	7d	7d
0.0	40	76	321	37.1	0.347	0.334	0.397	0.381
4.7	42	93	349	46.0	0.347	0.372	0.402	0.405
9.4	41	96	340	46.2	0.359	0.392	0.405	0.412
19.0	41	95	294	38.1	0.359	0.390	0.384	0.385
38.0	43	88	262	30.8	0.383	0.370	0.368	0.354
77.0	32	60	121	12.0	0.235	0.275	0.257	0.220
157	31	47	61	5.7	0.195	0.201	0.152	0.106
EC_{50}					159	165	116	**90 (75-108)**
Steepness					1.09	1.05	1.06	**1.18 (.75-1.62)**

(7) Fairchild et al. 1994, 1998

The authors assessed the effects of four herbicides on plant growth using 4-d tests with *Lemna minor* and 14-d tests with *Ceratophyllum dermersum*, *Elodea canadensis*, *Myriophyllum heterophyllum*, and *Najas* sp. Temperature was 25 C and light was 60 $\mu E/m^2/s$Concentrations were not measured in exposure chambers, but the stock concentrations were verified. The 1994 report provided detailed biomass measurements absent in the 1998 journal article.

Lemna Initial frond counts were 12 in each replicate and final frond counts are listed in the following table. The limited duration resulted in limited growth (barely 2-fold in the control) that makes these results rather uncertain, particularly based on frond counts.

Nominal Atrazine Conc (µg/L)	Final frond counts in replicates	SGRs (1/d)
0	34,26,23	0.260,0.193,0.163
37.5	25,25,19	0.184,0.115,0.163
75	19,20,15	0.128,0.056,0.101
150	15,17,20	0.087,0.128,0.092
300	16,18,22	0.101,0.152,0.110

600	12,14,14	0.000,0.038,0.026
EC_{50} (µg/L)		**114 (34-390)**
Steepness		**0.42 (0.06-0.79)**

Najas: Replicates were created by placing natural pond sediments from *Najas* beds in beakers, from which plants germinated. Plants were grown for approximately 2 weeks to approximately 3 cm in height, at which time the 14-d chemical exposure began. After the exposure, plants were sieved and wet weights were determined. Initial wet weights were not determined, but based on the similarity in the average weights in the highest three treatments (following table) it was assumed that these treatments had zero net growth and SGRs were estimated based on an initial wet weight of 69.5 mg, the overall average final weight of these treatments. Given the number of replicates with lower final weights, the initial weights obviously varied considerably across replicates, but by basing SGR on the mean weight across replicates, this variability is reduced enough to produce a clear dose-response. To the extent that the highest three treatments did not have zero net growth the estimated EC50 will be biased, but substantial bias would be unlikely because (a) if substantial positive growth was occurring a concentration effect should be evident and (b) if substantial negative growth was occurring this would imply a high initial weight incompatible with the information on control growth (i.e. a disproportionate amount of control growth in the two weeks prior to exposure compared to the 2 weeks of exposure).

Nominal Atrazine Conc (µg/L)	Final wwt for replicates (mg)	Final mean wwt for treatment (mg)	SGRs (1/d)
Control	306,111,122	180	0.068
Solvent Control	285,168,57	170	0.064
8.4	66,170,185	140	0.050
18.8	164,68,57	96	0.023
37.5	57,91,55	68	-0.001
75	65,7,137	70	+0.001
150	49,75,90	71	+0.002
EC_{50} (µg/L)			**14.5 (12.3-17.2)**
Steepness			**1.67 (1.00-2.33)**

Ceratophyllum: The authors provided wet weights for each replicate at 0, 7, and 14 d, allowing calculation of SGRs and regression analysis of these SGRs to determine the EC_{50} and steepness of the SGR vs concentration relationship. There was nearly a doubling of weight in the controls over the 14-d, allowing sufficient growth so that effects were apparent and could be quantified.

Nominal Atrazine Conc (µg/L)	Initial wwt for replicates (mg)	Final (14 d) wwt for replicates (mg)	SGR for replicates (1/d)

Control	1578,1202,1730	2292,2409,2735	0.027,0.050,0.033
Solvent Control	1310,1746,1622	2010,2965,2477	0.031,0.038,0.030
18.8	1209,937,1232	1476,1262,1798	0.014,0.021,0.027
37.5	1960,1777,1089	2281,2076,1378	0.011,0.011,0.017
75	2649,1062,2420	2410,1078,2434	-0.007,0.001,0.000
150	1362,1322,1482	1454,1446,1415	0.005,0.006,-0.003
300	1166,1516,878	1102,1563,1023	-0.004,0.002,0.010
EC_{50} (μg/L)			**24 (14-42)**
Steepness			**0.81 (0.12-1.50)**

Myriophyllum: The authors provided wet weights for each replicate at 0, 7, and 14 d, allowing calculation of the SGR for each replicate. However, the growth in controls and in NOECs was too small and variable for good quantification of effects on SGR. At day 14 (table below), the weight gain of individual replicates varied from -4-16% (average 8%) in the control, 1-31% (13%) in the solvent control, from 11-16% (15%) at 37.5 μg/L, and 2-26% (15%) at 75 μg/L. In addition, at day 7, the weight gains were 12-17% (15%) in the controls, 25-33% (28%) in the solvent controls, 13-16% (13%) at 37.5 μg/L, and 6-21% (11%) at 75 μg/L. These data illustrate not just a small amount of growth and great variability relative to the average net growth, but also no or negative growth in most replicates during the second week, which the authors also noted in other experiments. In addition, there is an inconsistency between the 7- and 14-d data in that the 14-d data show no difference among the controls and the two lowest concentrations, whereas the 7-d data indicate better growth in the solvent controls relative to the control without solvent and the two lowest concentrations. Although there are clear effects at 150 μg/L and above, there is not a good reference against which to quantify effects on the SGR. This underscores the requirement in the protocol that control growth be large and consistent enough to quantify ECs with reasonable precision. The most that can be inferred from this test is that 37.5 and 75μg/L are apparently NOECs and the SGR EC_{50} is probably ≈<150 μg/L.

Nominal Atrazine Conc (μg/L)	Initial wwt for replicates (mg)	Final (14 d) wwt for replicates (mg)	SGR for replicates (1/d)	SGR for treatment (1/d)
Control	3330,4547,3200	3696,4379,3712	0.007,-0.003,0.011	0.005
Solvent Control	3137,3767,3817	3184,3981,5017	0.001,0.004,0.020	0.008
37.5	2600,3077,3084	3021,3402,3603	0.011,0.007,0.011	0.010
75	3046,2872,4122	3895,3382,4197	0.018,0.012,0.001	0.010
150	3262,3854,4414	3782,3726,4454	0.011,-0.002,0.001	0.003
300	3559,3039,2756	3359,2074,2829	-0.004,-0.027,0.002	-0.010
600	2812,3748,3341	1877,3363,2992	-0.029,-0.008,-0.008	-0.015
EC_{50} (μg/L)				**<≈150**
Steepness				

Elodea: The authors provided both wet weights for each replicate at 0, 7, and 14 d, allowing calculation of the SGR for each replicate. However, as for *Myriophyllum*, the control growth was very small, averaging only about 15% over the two weeks. Although, this growth was not as variable as for *Myriophyllum*, it still is a questionable reference against which to quantify effects on SGRs. In addition, the lowest treatment concentration produced no growth on average, and negative growth became progressively greater at higher concentrations, so that ECs for SGR could not be quantified even if the controls were good references for quantifying the SGR. The most that can be inferred from this test is that the SGR EC_{50} is <38 μg/L, although even this might be confounded by the low control growth.

Nominal Atrazine Conc (μg/L)	Initial wwt for replicates (mg)	Final (14 d) wwt for replicates (mg)	SGR for replicates (1/d)	SGR for treatment
Control	4820,5564,6866	5949,6345,7802	0.015,0.009,0.009	0.014
Solvent Control	5554,5672,6624	6336,6140,7016	0.009,0.006,0.004	0.008
37.5	7146,3370,5500	7258,3232,5556	0.001,-0.003,0.001	0.001
75	6028,5477,6477	5435,5178,6478	-0.007,-0.004,0.000	-0.002
150	4941,4929,4992	4778,4851,5554	-0.002,-0.001,0.007	-0.002
300	6080,5937,5398	5575,5543,5087	-0.006,-0.005,-0.004	-0.004
600	6902,7160,6200	3960,6302,5605	-0.040,-0.009,-0.007	-0.018
EC_{50} (μg/L)				**<37.5**
Steepness				

(8) Fairchild et al. 1995, 1997

The authors conducted 4-d tests of *Lemna minor* growth at multiple atrazine concentrations (as well as 15 other herbicides). Concentrations were not measured. For *Lemna*, the reported EC_{50} of 153 μg/L was based on growth (frond count basis), and insufficient information was provided to convert this to a growth rate basis. Based on a control growth rate of 0.21/d for identical methodology used above by Fairchild et al. (1994, 1998) this EC_{50} would correspond to an EC_{82}. Because this extrapolation was greater than allowed in the protocol, this data just indicate that the SGR EC_{50} is <153 μg/L, which does not contradict the results of Fairchild et al. (1994, 1998).

(9) Kirby and Sheahan 1994

The authors conducted a 10-d test of the growth of *Lemna minor* at multiple atrazine concentrations; concentrations were measured. Temperature was 25 C and light intensity was 3500 lux. The authors only reported EC50s based on final biomass, without any information on specific treatments, growth rates, etc. The initial biomass was 10 fronds and growth was quantified by chlorophyll, frond count, and fresh weight, with the respective EC_{50}s being 56, 60, and 62 μg/L. Using the average SGR_C from other studies with *Lemna* (0.27/d, range 0.21-0.38/d), the EC_{50} for frond count would correspond to an EC_{25} for SGR. Using the average steepness for SGR vs. concentration from other studies with *Lemna* (1.0 for frond count increase,

1.4 for weight increase), the SGR EC_{50} would then be **105 μg/L** based on frond count and **95 μg/L** based on weight.

(10) University of Mississippi 1991

The authors evaluated growth of *Lemna gibba* (14 d), and *Elodea canadensis* (10 d) at multiple atrazine concentrations. These assays entailed methodological and performance problems that precluded their use, especially for determining SGR-based ECs. Chlorophyll measurements were erratic in addition to being not accepted in the protocol used here. For *Lemna*, both frond counts and weights were measured, but frond counts indicated poor control growth (an SGR of 0.1/d, compared to 0.2-0.4/d in other studies), no initial weights were given, and final weights had poor precision. For *Elodea*, final dry weights did show a substantial effect of atrazine, but initial weights were not given, so that growth could not be assessed either as a rate or an absolute amount. For both species, atrazine effects were evident at 100 μg/L, but the next lower and higher concentration was 10-fold different (10 and 1000 μg/L) , precluding any good characterization of dose-response.

(11) Forney and Davis 1981; Davis 1980, Forney 1980

The authors evaluated growth of *Elodea canadensis*, *Myriophyllum spicatum*, *Potamogeton perfoliatus*, and *Vallisneria americana* in exposures of 3-9 weeks to multiple atrazine concentrations. Depending on the experiment and test species, light varied from 3 to 170 $\mu E/m^2/s$ (14/10 h photoperiod) and temperature was 20-30 C. Unfortunately, most of the evaluations were of shoot length increase, which as discussed above is a questionable surrogate for growth. In three instances, useful information regarding the SGR EC_{50} could be obtained:

For *Potamogeton*, in one experiment, dry weight was measured in addition to shoot length. However, the nature of the weight measurements was unclear (gross weight vs. growth, how much of plant included) and the authors noted that food reserves in the tuber used to sprout *Potamogeton* would partially mask herbicide effects, so that these weight measurements would overestimate ECs. This experiment also showed atrazine-dependent mortality at concentrations of 32 μg/L and above. The following table shows the average dry weight of plants (at death or end of test for survivors), the percent survival, and the product of dry weight and survival as an estimate of live biomass at the end of the study. For issues regarding weight effects already noted, this product might still underestimate biomass production, but was considered adequately informative of atrazine effects on the SGR of a population of this plant. A regression analysis was thus conducted on this product and used for the compilation.

Nominal Atrazine Conc (μg/L)	% of Control Dry Weight	% Survival	% of Control Biomass
0	100	100	100
10	86	100	86
32	86	73	63
100	74	62	46

320	55	0	0
EC_{50} (μg/L)			**63**
Steepness			**0.69**

For *Vallisneria*, leaf length was measured and was used as a surrogate for growth because it would be less susceptible than shoot length to elongation with little or no weight increase. Even with this acceptance, most data could not be used because the authors noted that effects of atrazine were not evident early in the experiments, likely due to food reserves in the tubers, and that some experiments had light intensities high enough to inhibit leaf growth in favor of tuber and lateral shoot development. Thus, analysis here was restricted to the latter part of one test that the authors reported as being most informative about atrazine effects. The following table provides the percentage increase in leaf length during the last week of this experiment, which should be approximately proportional to the SGR. In another experiment with insufficient data for analysis here, there was information on the ratio of plant weight to leaf length as a function of atrazine, which did indicate some thinning of the leaves due to atrazine. The following table includes those ratios, which provided a basis for estimating weight based on leaf length (only three measured values – so interpolated value used for 32 μg/L and possible extrapolated values for 1000 μg/L). This resulted in a decrease in the SGR EC50 of about 28%.

Nominal Atrazine Conc (μg/L)	% Increase in Leaf Length in Week 6	Dry Weight/ Leaf Length (fraction of control)	Estimated % Increase in Weight
0	14.3	1.00	14.3
32	9.8	*0.97*	9.5
100	10.2	0.94	9.6
320	5.9	0.82	4.8
1000	3.6	*0.7-0.8*	*2.5-2.9*
EC_{50} (μg/L)	195		**140-141**
Steepness	0.36		**0.39-0.41**

For *Elodea*, in one experiment dry weight increase was measured. The following table provides these data. Because initial and final dry weights weren't provided, SGRs cannot be calculated, but the slow growth rates of these plants should make the net increase proportional to SGR. Because of the widely space concentrations, the estimated parameters are uncertain, but clearly indicate the SGR EC50 to be less than 100 μg/L.

Nominal Atrazine Conc (μg/L)	Average Increase in Plant Dry Wt. (mg)
0	37
10	28
100	17
1000	11
EC_{50} (μg/L)	**65**

Steepness	**0.28**

(10) Hinman 1989

The author tested the effects of atrazine on both root and shoot growth of *Hydrilla verticillata* in both water and sediment exposures (14 d). Concentrations were nominal, light was 40-50 $\mu E/m^2/s$, and temperature was 25 C. Both shoot and root growth was monitored by increase in length. Increases in shoot length are subject to questions about elongation without increasing weight, but this is not true for root growth, which should still be an indicator of atrazine effects on primary production. The following table compares the data on root and shoot growth for the water-based exposures. Shoot lengths do indicate a higher threshold for effects, but then a steeper decline, with the EC50 being about 80% higher than for root length.

Nominal Atrazine Conc (µg/L)	Shoot Length Increase (% of Control)	Root Length Increase (% of Control)
0	100	100
16	97	98
80	127	71
160	83	25
800	5	25
1600	5	8
EC_{50} (µg/L)	222	**118**
Steepness	2.26	**0.6**

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APPENDIX B.

EXPERIMENTAL ECOSYSTEM DATA

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Table B1. Summary of experimental ecosystem studies used in development of $PATI_{LOC}$. ID# identifies treatment and cross-references exposure time-series provided in Table B2. Effect is binary (yes/no) regarding whether substantial impact on plant community occurred.

ID #	Duration (d)	Initial Conc. (μg/L Atrazine)	Significant Effect?	Reference
1	365	500	Y	Carney 1983; Kettle et al. 1987; deNoyelles et al. 1989; deNoyelles et al. 1994
2	365	20	Y	Carney 1983; Kettle et al. 1987; deNoyelles et al. 1989; deNoyelles et al. 1994, deNoyelles & Kettle 1980, Dewey 1986
3	63	500	Y	deNoyelles et al. 1982; deNoyelles et al. 1989
4	365	100	Y	deNoyelles et al. 1989 Carney 1983
5	340	200	Y	deNoyelles et al. 1989 Carney 1983
7	56	80	Y	Hamilton et al. 1987
8	56	140	Y	Hamilton et al. 1987
9	96	100	Y	Hamilton et al. 1988
10	96	100	Y	Herman et al. 1986; Hamilton et al. 1989
13	53	430	Y	Stay et al. 1985
14	53	820	Y	Stay et al. 1985
15	53	3980	Y	Stay et al. 1985
17	7	100	Y	Brockway et al. 1984
18	12	500	Y	Brockway et al. 1984
19	12	5000	Y	Brockway et al. 1984
22	15	15	Y	Detenback et al. 1996
23	43	25	Y	Detenback et al. 1996
24	32	50	Y	Detenback et al. 1996
25	17	79	Y	Detenback et al. 1996
26	14	100	Y	Hamala and Kollig 1985
27	30	1000	Y	Johnson 1986
28	21	10	Y	Kosinski 1984; Kosinski and Merkle 1984
29	21	1000	Y	Kosinski 1984; Kosinski and Merkle 1984
30	21	10000	Y	Kosinski 1984; Kosinski and Merkle 1984
31	12	24	Y	Krieger et al. 1988
32	12	134	Y	Krieger et al. 1988
33	7	10000	Y	Moorhead and Kosinski 1986

Table B1 (continued).

ID #	Duration (d)	Initial Conc. (μg/L Atrazine)	Significant Effect?	Reference
34	21	337	Y	Pratt et al. 1988
35	42	204	Y	Stay et al. 1989
36	42	500	Y	Stay et al. 1989
37	42	1000	Y	Stay et al. 1989
38	42	5000	Y	Stay et al. 1989
39	55	50	Y	Brockway et al. 1984
40	15	100	Y	Brockway et al. 1984
41	360	100	Y	deNoyelles et al. 1989
42	360	200	Y	deNoyelles et al. 1989
44	21	100	Y	Kosinski 1984; Kosinski and Merkle 1984
45	7	100	Y	Moorhead and Kosinski 1986
46	7	1000	Y	Moorhead and Kosinski 1986
47	53	53	Y	Stay et al. 1985
48	53	84	Y	Stay et al. 1985
49	53	170	Y	Stay et al. 1985
50	42	100	Y	Stay et al. 1989
51	12	50	Y	Brockway et al. 1984
52	63	20	Y	deNoyelles et al. 1982; deNoyelles et al. 1989
53	30	10	N	Johnson 1986
54	30	100	N	Johnson 1986
58	18	1	Y	Lampert et al 1989
58b	42	0.1	Y	Lampert et al 1989
59	21	32	Y	Pratt et al. 1988
60	21	110	Y	Pratt et al. 1988
61	42	20	N	Stay et al. 1989
62	35	5	N	van den Brink et al. 1995
63	7	0.5	N	Brockway et al. 1984
64	7	5	N	Brockway et al. 1984
65	29	0.5	N	Brockway et al. 1984
66	70	5	N	Brockway et al. 1984

Table B1 (continued).

ID #	Duration (d)	Initial Conc. (μg/L Atrazine)	Significant Effect?	Reference
67	14	5	N	Gruessner and Watzin 1996
68	20	1	N	Gustavson and Wängberg 1995
69	20	20	N	Gustavson and Wängberg 1995
70	20	10	N	Gustavson and Wängberg 1995
71	28	2	N	Jurgensen and Hoagland 1990
72	28	30	N	Jurgensen and Hoagland 1990
73	28	100	N	Jurgensen and Hoagland 1990
75	30	25	N	Lynch et al. 1985
76	21	3.2	N	Pratt et al. 1988
77	21	10	N	Pratt et al. 1988
78	30	25	Y	Rohr and Crumrine, 2005
79	28	117	Y	Rohr et al., 2008
80	36	6.4	N	Relyea, 2009
81	173	84	Y	Knauert et al., 2008; Knauert et al., 2009
82	23	10	Y	Berard et al. 1999a, Berard et al. 1999b, Berard and Benninghoff 2001, Sequin et al. 2001b, Leboulanger et al. 2001
83	40	30	N	Seguin et al. 2001a
84	40	2	N	Seguin et al. 2001a
85	40	30	Y	Seguin et al. 2001b
86	40	2	Y	Seguin et al. 2001b
87	25	30	Y	Seguin et al. 2002
88	7	148	Y	Downing et al. 2004
89	7	24.3	Y	Downing et al. 2004
90	25	207	N	Boone and James 2003
95	51	20	N	Diana et al. 2000
96	51	196	Y	Diana et al. 2000
97	51	2036	Y	Diana et al. 2000
98	42	25	N	McGregor et al. 2008
99	42	50	N	McGregor et al. 2008
100	42	100	Y	McGregor et al. 2008
101	42	250	Y	McGregor et al. 2008

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Table B2. Atrazine exposure time-series for experimental ecosystem treatments, with ID# as specified in Table B1.

ID #1		ID#2		ID#3		ID#4		ID#5		ID#7		ID#8		ID#9	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	500	0	20.0	0	500	0	100	0	200	1	80	1	140	1	100
10	525	10	16.0	2	490	10	90	20	190	3	79	56	110	5	117
20	490	20	16.0	25	465	20	85	40	120	5	78			14	108
40	350	40	16.0	30	453	40	90	60	160	7	78			20	107
70	490	70	15.0	55	390	70	80	70	140	9	77			24	87
100	400	100	12.0	63	360	100	75	80	150	11	76			34	105
130	400	130	14.0			130	70	105	120	13	76			37	142
180	375	180	15.0			180	70	130	120	15	75			42	148
285	250	285	7.0			285	35	160	110	17	75			54	132
330	200	330	5.0			330	30	190	140	19	74			68	115
365	160	365	4.0			365	25	220	120	21	73			96	53
								250	100	23	73				
								290	90	25	72				
								340	50	27	71				
										29	71				
										31	70				
										33	70				
										35	69				
										37	69				
										39	68				
										41	67				
										43	67				
										45	66				
										47	66				
										49	65				
										51	65				
										53	64				
										55	64				

Table B2, Page 2.

ID #10		ID#13		ID#14		ID#15		ID#17		ID#18		ID#19		ID#22	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
1	100	0	430	0	820	0	3980	0	100	0	500	0	5000	1	15.0
5	117	21	264	21	505	21	1890	1	100	1	498	1	4979	2	13.6
14	108	46	223	46	443	46	1390	2	99	2	496	2	4958	3	12.9
20	107	53	198	53	417	53	1540	3	99	3	494	3	4937	4	12.3
24	87							4	98	4	492	4	4917	5	11.7
34	105							5	98	5	490	5	4896	6	11.1
37	142							6	98	6	488	6	4876	7	10.6
42	148							7	97	7	486	7	4855	8	10.1
54	132									8	484	8	4835	9	9.6
68	115									9	481	9	4815	10	9.1
96	53									10	479	10	4794	11	8.7
										11	477	11	4774	12	8.3
										12	475	12	4754	13	7.9
														14	7.5
														15	7.1

Table B2, Page 3.

ID #23		ID#24		ID#25		ID#26		ID#27		ID#28		ID#29		ID#30	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
1	25.1	1	50	1	79	0	100	0	1000	1	10.0	1	1000	1	10000
3	21.6	3	43	2	72	14	100	2	992	21	10.0	2	648	2	6484
5	19.6	5	39	3	68			4	983			3	522	3	5221
7	17.7	7	35	4	65			6	975			4	420	4	4205
9	16.1	9	32	5	62			8	967			5	339	5	3386
11	14.6	11	29	6	59			10	959			6	273	6	2726
13	13.2	13	26	7	56			12	951			7	220	7	2195
15	11.9	15	24	8	53			14	943			8	177	8	1768
17	10.8	17	21	9	51			16	935			9	142	9	1424
19	9.8	19	19	10	48			18	927			10	115	10	1146
21	8.9	21	18	11	46			20	919			11	92	11	923
23	8.0	23	16	12	44			22	912			12	74	12	743
25	7.3	25	14	13	42			24	904			13	60	13	599
27	6.6	27	13	14	40			26	897			14	48	14	482
29	6.0	29	12	15	38			28	889			15	39	15	388
31	5.4	31	11	16	36			30	882			16	31	16	313
33	4.9			17	34							17	25	17	252
35	4.4											18	20	18	203
37	4.0											19	16	19	163
39	3.6											20	13	20	131
41	3.3											21	11	21	106
43	3.0														

Table B2, Page 4.

ID #31		ID#32		ID#33		ID#34		ID#35		ID#36		ID#37		ID#38	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	24.0	0	134	0	10000	0	337	1	204	1	492	1	961	1	4929
12	24.0	12	134	1	9958	21	337	3	199	3	474	3	931	3	4806
				2	9916			5	196	5	463	5	918	5	4758
				3	9875			7	193	7	452	7	907	7	4710
				4	9833			9	190	9	441	9	895	9	4662
				5	9792			11	187	11	430	11	883	11	4615
				6	9751			13	184	13	420	13	872	13	4569
				7	9710			15	181	15	410	15	860	15	4523
								17	178	17	400	17	849	17	4477
								19	175	19	390	19	838	19	4432
								21	172	21	381	21	827	21	4388
								23	169	23	372	23	816	23	4344
								25	167	25	363	25	806	25	4300
								27	164	27	354	27	795	27	4257
								29	161	29	346	29	785	29	4214
								31	159	31	337	31	775	31	4171
								33	156	33	329	33	765	33	4129
								35	154	35	321	35	755	35	4088
								37	151	37	314	37	745	37	4047
								39	149	39	306	39	735	39	4006
								41	146	41	299	41	726	41	3966

Table B2, Page 5.

ID #39		ID#40		ID#41		ID#42		ID#44		ID#45		ID#46		ID#47	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	50	0	100	0	100	0	200	1	100	1	100	1	1000	0	52
55	50	15	100	180	70	180	140	2	65	2	99	2	992	21	48
				360	25	360	50	3	52	3	99	3	988	46	41
								4	42	4	98	4	983	53	34
								5	34	5	98	5	979		
								6	27	6	98	6	975		
								7	22	7	97	7	971		
								8	18						
								9	14						
								10	12						
								11	9						
								12	7						
								13	6						
								14	5						
								15	4						
								16	3						
								17	3						
								18	2						
								19	2						
								20	1						

Table B2, Page 6.

ID #48		ID#49		ID#50		ID#51		ID#52		ID#53		ID#54		ID#58	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	84	0	169	1	100	0	50	1	20.0	0	10.0	0	100	0	1.0
21	63	21	114	3	97	1	50	2	19.5	2	9.9	2	99	1	1.0
46	60	46	95	5	96	2	50	25	18.0	4	9.8	4	98	2	1.0
53	51	53	98	7	94	3	49	30	17.0	6	9.8	6	98	3	1.0
				9	92	4	49	55	15.0	8	9.7	8	97	4	1.0
				11	91	5	49	63	14.5	10	9.6	10	96	5	1.0
				13	89	6	49			12	9.5	12	95	6	1.0
				15	88	7	49			14	9.4	14	94	7	1.0
				17	86	8	48			16	9.3	16	94	8	1.0
				19	85	9	48			18	9.3	18	93	9	1.0
				21	83	10	48			20	9.2	20	92	10	1.0
				23	82	11	48			22	9.1	22	91	11	1.0
				25	80	12	48			24	9.0	24	90	12	1.0
				27	79					26	9.0	26	90	13	0.9
				29	78					28	8.9	28	89	14	0.9
				31	76					30	8.8	30	88	15	0.9
				33	75									16	0.9
				35	74									17	0.9
				37	72									18	0.9
				39	71										
				41	70										

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Table B2, Page 7.

ID #58b		ID#59		ID#60		ID#61		ID#62		ID#63		ID#64		ID#65	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	0.1	0	32	0	110	1	17.7	0	5.0	1	0.5	1	5.0	0	0.5
42	0.1	10	32	10	110	3	17.4	35	5.0	2	0.5	2	5.0	29	0.5
		21	32	21	110	5	17.1			3	0.5	3	4.9		
						7	16.9			4	0.5	4	4.9		
						9	16.7			5	0.5	5	4.9		
						11	16.5			6	0.5	6	4.9		
						13	16.3			7	0.5	7	4.9		
						15	16.1								
						17	15.9								
						19	15.7								
						21	15.5								
						23	15.3								
						25	15.1								
						27	14.9								
						29	14.7								
						31	14.5								
						33	14.3								
						35	14.1								
						37	14.0								
						39	13.8								
						41	13.6								

Table B2, Page 8.

ID #66		ID#67		ID#68		ID#69		ID#70		ID#71		ID#72		ID#73	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	5.0	1	4.7	1	1.0	1	20.0	1	10.0	1	2.0	1	30	1	100
70	5.0	5	3.6	2	1.0	2	19.8	2	9.9	2	1.6	2	23	2	78
		10	1.2	3	1.0	3	19.7	3	9.9	3	0.0	3	0	3	0
		14	1.2	4	1.0	4	19.7	4	9.8	4	0.0	4	0	4	0
				5	1.0	5	19.6	5	9.8	5	0.0	5	0	5	0
				6	1.0	6	19.5	6	9.8	6	0.0	6	0	6	0
				7	1.0	7	19.4	7	9.7	7	0.0	7	0	7	0
				8	1.0	8	19.3	8	9.7	8	0.0	8	0	8	0
				9	1.0	9	19.3	9	9.6	9	0.0	9	0	9	0
				10	1.0	10	19.2	10	9.6	10	0.0	10	0	10	0
				11	0.9	11	19.1	11	9.5	11	0.0	11	0	11	0
				12	0.9	12	19.0	12	9.5	12	0.0	12	0	12	0
				13	0.9	13	18.9	13	9.5	13	0.0	13	0	13	0
				14	0.9	14	18.9	14	9.4	14	2.0	14	30	14	100
				15	0.9	15	18.8	15	9.4	15	1.6	15	23	15	78
				16	0.9	16	18.7	16	9.3	16	0.0	16	0	16	0
				17	0.9	17	18.6	17	9.3	17	0.0	17	0	17	0
				18	0.9	18	18.5	18	9.3	18	0.0	18	0	18	0
				19	0.9	19	18.5	19	9.2	19	0.0	19	0	19	0
				20	0.9	20	18.4	20	9.2	20	0.0	20	0	20	0
										21	0.0	21	0	21	0
										22	0.0	22	0	22	0
										23	0.0	23	0	23	0
										24	0.0	24	0	24	0
										25	0.0	25	0	25	0
										26	0.0	26	0	26	0
										27	0.0	27	0	27	0
										28	0.0	28	0	28	0

Table B2, Page 9.

ID #75		ID#76		ID#77		ID#78		ID#79		ID#80		ID#81		ID#82	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
0	25.0	0	3.2	0	10.0	1	25.0	1	117	1	6.4	1	84	1	10.0
30	25.0	21	3.2	21	10.0	2	24.8	2	116	3	6.3	7	80	2	9.9
						3	24.7	3	116	5	6.3	13	77	3	9.9
						4	24.6	4	115	7	6.2	19	74	4	9.8
						5	24.5	5	115	9	6.2	25	78	5	9.8
						6	24.4	6	114	11	6.1	31	75	6	9.8
						7	24.3	7	114	13	6.1	37	72	7	9.7
						8	24.2	8	113	15	6.0	43	69	8	9.7
						9	24.1	9	113	17	6.0	49	66	9	9.6
						10	24.0	10	112	19	5.9	55	64	10	9.6
						11	23.9	11	112	21	5.9	61	61	11	9.5
						12	23.8	12	111	23	5.8	67	59	12	9.5
						13	23.7	13	111	25	5.8	73	57	13	9.5
						14	23.6	14	110	27	5.7	79	55	14	9.4
						15	48.5	15	110	29	5.7	85	53	15	9.4
						16	48.3	16	109	31	5.6	91	51	16	9.3
						17	48.1	17	109	33	5.6	97	49	17	9.3
						18	47.9	18	109	35	5.5	103	47	18	9.3
						19	47.7	19	108			109	45	19	9.2
						20	47.5	20	108			115	43	20	9.2
						21	47.3	21	107			121	42	21	9.2
						22	47.1	22	107			127	40	22	9.2
						23	46.9	23	106			133	39	23	9.2
						24	46.7	24	106			139	37		
						25	46.5	25	105			145	36		
						26	46.3	26	105			151	34		
						27	46.1	27	105			157	33		
						28	45.9	28	104			163	32		
						29	45.7					169	31		
						30	45.5								

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ID #83		ID#84		ID#85		ID#86		ID#87		ID#87		ID#89		ID#90	
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)
1	30	1	2.0	1	30	1	2.0	1	30	1	148	1	24.3	1	207
3	30	3	2.0	3	30	3	2.0	2	30	2	127	2	18.3	3	170
5	29	5	2.0	5	29	5	2.0	3	30	3	120	3	20.7	5	148
7	29	7	1.9	7	29	7	1.9	4	30	4	112	4	19.6	7	130
9	29	9	1.9	9	29	9	1.9	5	29	5	105	5	18.6	9	114
11	29	11	1.9	11	29	11	1.9	6	29	6	98	6	17.6	11	99
13	28	13	1.9	13	28	13	1.9	7	29	7	88	7	15.4	13	87
15	28	15	1.9	15	28	15	1.9	8	29					15	76
17	28	17	1.9	17	28	17	1.9	9	29					17	67
19	28	19	1.8	19	28	19	1.8	10	29					19	58
21	28	21	1.8	21	28	21	1.8	11	29					21	51
23	27	23	1.8	23	27	23	1.8	12	29					23	45
25	27	25	1.8	25	27	25	1.8	13	28					25	39
27	27	27	1.8	27	27	27	1.8	14	28					27	34
29	27	29	1.8	29	27	29	1.8	15	28					29	30
31	26	31	1.8	31	26	31	1.8	16	28					31	26
33	26	33	1.7	33	26	33	1.7	17	28					33	23
35	26	35	1.7	35	26	35	1.7	18	28					35	20
37	26	37	1.7	37	26	37	1.7	19	28					37	18
39	26	39	1.7	39	26	39	1.7	20	28					39	15
								21	28					41	14
								22	27					43	12
								23	27					45	10
								24	27					47	9
								25	27					49	8
														51	7
														53	6
														55	5

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ID #95		ID#96		ID#97		ID#98		ID#99		ID#100		ID#101			
Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)	Time (d)	Conc (μg/L)		
1	20.1	1	196	1	2036	1	24.5	1	50	1	104	1	248		
3	19.5	3	193	3	1986	42	24.5	42	50	42	104	42	248		
5	19.0	5	191	5	1954										
7	18.6	7	189	7	1922										
9	18.2	9	188	9	1890										
11	17.8	11	186	11	1859										
13	17.4	13	184	13	1829										
15	17.0	15	183	15	1799										
17	16.6	17	181	17	1769										
19	16.3	19	180	19	1740										
21	15.9	21	178	21	1712										
23	15.6	23	176	23	1684										
25	15.2	25	175	25	1656										
27	14.9	27	173	27	1629										
29	14.6	29	172	29	1603										
31	14.2	31	170	31	1576										
33	13.9	33	169	33	1551										
35	13.6	35	167	35	1525										
37	13.3	37	166	37	1500										
39	13.0	39	164	39	1476										
41	12.7	41	163	41	1452										
43	12.4	43	161	43	1428										
45	12.2	45	160	45	1404										
47	11.9	47	158	47	1381										
49	11.6	49	157	49	1359										
51	11.4	51	155	51	1337										

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